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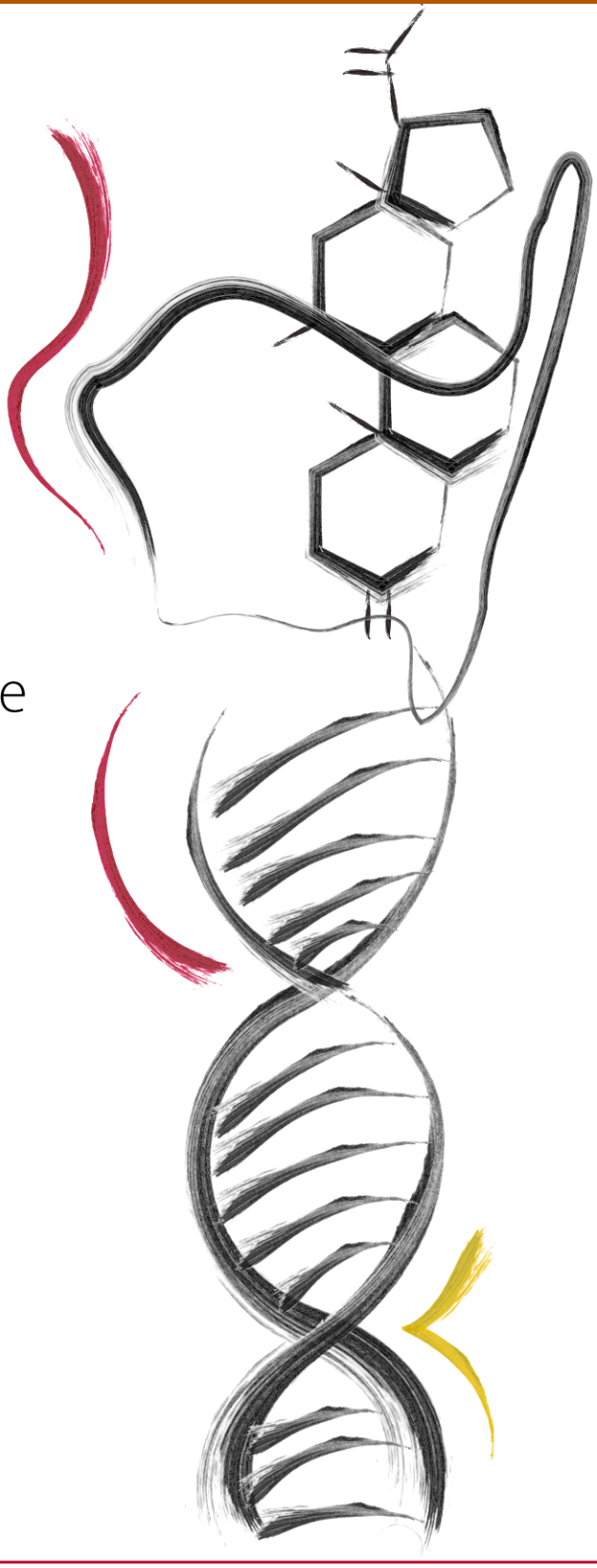
The role of stress in vocal symptoms:

A biologically informed perspective



Sofia Holmqvist Jämsén

The role of stress in vocal symptoms: A biologically informed perspective

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"Our facts must be correct. Our theories need not be,
if they help us discover important new facts."

Hans Selye

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I Rantala, L.M., Hakala, S., Holmqvist, S., & Sala, S. (2012). Connections between voice ergonomic risk factors and voice symptoms, voice handicap and respiratory tract diseases. *Journal of Voice*, *16*, 819.e13-e20. doi: 10.1016/j.jvoice.2012.06.001

II Holmqvist, S., Santtila, P., Lindström, E., Sala, E., & Simberg, S. (2013). The association between possible stress markers and vocal symptoms. *Journal of Voice, 27,* 787.e1-787.e10. doi:10.1016/j.jvoice.2013.06.012

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AUTHORS CONTRIBUTION

Study I: The author participated in planning of the research project and assessment methods. Furthermore, the author took active part in the research group and participated in the data collection and assessments. She also contributed to the writing of the manuscript.

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SAMMANFATTNING

Bakgrund: Röstsymtom och röststörningar inverkar på kommunikation och mellanmänsklig interaktion och kan resultera i begränsningar av individens aktivitet och känsla av delaktighet. I flera studier där etiologiska faktorer till röstsymtom eller röststörningar har undersökts har stress visat sig vara en bidragande faktor till röstsymtom. Även om många studier har identifierat stress som en riskfaktor är sambandet mellan stress och förekomsten av röstsymtom fortfarande oklart. Endast ett fåtal studier har undersökt inverkan av fysiologiska effekter av stress på förekomsten av röstsymtom eller röststörningar. Avhandlingen syftar till att undersöka sambandet mellan stress och röstsymtom med fokus på möjliga somatiska och biologiska stressmarkörer samt potentiella genetiska faktorer relaterade till stress.

Syfte: Det övergripande syftet med avhandlingen var att undersöka sambandet mellan stress och röstsymtom hos personer i röstkrävande yrken, samt i ett populationsbaserat sampel. Mer specifikt var syftet att undersöka sambandet med hjälp av den biologiska stressmarkören kortisol samt att undersöka om möjliga stressrelaterade genetiska riskfaktorer kan förklara interindividuell variation i förekomsten av röstsymtom.

Deltagare: Två olika sampel användes. Det första bestod av 39 finländska lågstadielärare, med en könsfördelning på 8 män och 31 kvinnor. Röstergonomiska riskfaktorer i lärarnas arbetsmiljö bedömdes och lärarna fyllde i ett frågeformulär kring röst och röstrelaterad hälsa (Studie I). Det andra samplet bestod av ett populationsbaserat urval av manliga och kvinnliga finländska tvillingar och deras syskon, födda mellan åren 1961 och 1989. Totalt 555 män och 1173 kvinnor svarade på en enkät om röst och röstrelaterade riskfaktorer (Studie II). Utöver att besvara enkäten ombads deltagarna att ge salivprov för DNA- och hormonanalys. Av dem som hade svarat på enkäten var det sammanlagt 170 deltagare (49 män, 121 kvinnor) som gav salivprov för hormonanalys (Studie III) och 657 deltagare (219 män, 438 kvinnor) som gav salivprov för DNA-analys (Studie IV).

Metoder: Från samplet av lågstadielärare (N = 39) erhölls enkätuppgifter angående röstsymtom och röstergonomiska riskfaktorer, inklusive

förekomsten av stress. Från det populationsbaserade samplet erhölls enkätdata kring röstsymtom, röstergonomiska riskfaktorer och möjliga stressmarkörer (*N* = 1728). Dessutom mättes nivåerna av stresshormonet kortisol från salivprov i ett delsampel av det populationsbaserade urvalet (*n* = 170), för att analysera sambandet mellan kortisolnivå och förekomsten av röstsymtom. För att undersöka associationen mellan röstsymtom och genetiska varianter utfördes DNA-analys av erhållna salivprov (*n* = 657). Enbaspolymorfismer i två gener med konstaterat samband till stress och socioemotionellt beteende valdes ut för analys – oxytocinreceptorgenen (*OXTR*) och vasopressin 1A receptorgenen (*AVPR1A*). Eftersom kortisol, oxytocin och vasopressin är viktiga psykoneuroendokrinologiska komponenter i stressreaktionen, undersöktes också ifall möjliga associationer mellan enbaspolymorfismerna och röstsymtom kunde vara medierade genom kortisol.

Resultat: Resultaten från Studie I visade att lågstadielärare som kände sig stressade, rapporterade mer röstsymtom än lärare med mindre eller ingen stress. Stress var också den riskfaktor som hade starkast koppling till förekomsten av röstsymtom, även om korrelationernas styrka mellan de olika röstergonomiska riskfaktorerna och stress inte skiljde sig mycket åt. Resultaten från Studie II visade ett positivt samband mellan de mätta stressmarkörerna och röstsymtom. Också analysen med den biologiska stressmarkören kortisol visade en positiv association till röstsymtom (Studie III). Personer med högre kortisolnivåer rapporterade fler röstsymtom. Resultaten från Studie IV visade att enbaspolymorfismen (rs1587097) i *AVPR1A* hade en signifikant association med förekomsten av röstsymtom. Dessutom indikerade resultaten att de nominellt signifikanta effekterna av en *OXTR* polymorfism (rs2268493) på röstsymtom delvis kunde vara medierade av kortisol.

Slutsatser: Ett positivt samband mellan stress och röstsymtom observerades både i samplet av lågstadielärare och i det populationsbaserade samplet. Även om associationen mellan kortisol och röstsymtom var relativt svagt, är resultatet intressant eftersom kortisol är en biomarkör för den fysiologiska stressresponsen och därför ger en mer objektiv uppfattning kring huruvida

individer upplever stress utan att behöva förlita sig på självrapportering. Resultaten kring effekterna av *OXTR* och *AVPR1A* polymorfismerna bör tolkas med försiktighet, eftersom den specifika associationen mellan receptorgenerna och oxytocin- eller vasopressinnivåerna i hjärnan och i kroppen ännu inte är klarlagd. Resultaten från avhandlingen bidrar med ny kunskap kring associationen mellan röstsymtom, stress och kortisol samt genetisk variation i *OXTR* och *AVPR1A*. Resultaten kan bidra till att utveckla den kliniska behandlingen av röststörningar och även till utformningen av framtida studier inom röstforskningen.

SUMMARY

Background: Vocal impairment influences communication and can result in restriction of activity and participation. In several studies where etiological factors for vocal symptoms have been investigated, stress has been a contributing factor. Even though many studies have identified stress as a risk factor, the nature and strength of the association between stress and the occurrence of vocal symptoms is still unclear and the physiological role of stress in voice disorders remains understudied. The present thesis investigates the association between stress and vocal symptoms with a focus on possible somatic stress markers, biological stress markers and potential genetic factors related to stress.

Aims: The general aim of the thesis was to investigate the association between stress and vocal symptoms in a sample of occupational voice users, as well as in a population based sample. Specifically, the aim was to study this association using a biological stress marker, and to explore whether possible stress related genetic risk factors explain variation in vocal symptoms between individuals.

Participants: Two different samples were used. The first sample consisted of 39 Finnish elementary school teachers, with a gender distribution of 8 men and 31 women. The voice ergonomic risk factors in the teachers' work environment were assessed and the teachers responded to a questionnaire. The second sample consisted of a population-based sample of Finnish male and female twins and their siblings, born between 1961 and 1989. A total of 555 men and 1173 women responded to a questionnaire regarding voice and voice related risk factors. In addition to completing the questionnaire, the participants were asked to give saliva samples for DNA and hormone analysis. Of those who had responded to the questionnaire altogether 657 participants (219 men, 438 women) provided saliva samples for DNA analysis, and 170 participants (49 men, 121 women) provided saliva samples for hormone analysis.

Methods: Survey data of vocal symptoms and of voice ergonomic risk factors, one being stress, were obtained from the sample of teachers. Data on vocal

symptoms and possible stress markers were gathered from the population based sample survey. Additionally, the levels of the stress hormone cortisol were extracted from the samples of saliva in order to analyze whether there would be a positive association between levels of cortisol and vocal symptoms. For the analysis of possible effects of stress related genetic variants on vocal symptoms, polymorphisms in the oxytocin receptor gene (*OXTR*) and the vasopressin 1A receptor gene (*AVPR1A*) were chosen for analysis, since both genes have been associated with social behaviors and stress. Apart from analyzing effects of these polymorphisms on vocal symptoms, a mediational analysis was conducted to investigate whether possible effects of the polymorphisms on vocal symptoms could be mediated through cortisol, given the involvement of cortisol, oxytocin and vasopressin in the psychoneuroendocrinology of the stress reaction.

Results: Teachers who felt stressed reported more vocal symptoms than teachers with little or no stress. Stress was also the one risk factor with the strongest connection to occurrence of vocal complaints; however, the differences between the risk factors were not prominent. A positive association between possible stress markers and vocal symptoms was found in the population-based sample. A positive association to vocal symptoms was also found when using the biological stress marker of salivary cortisol. After controlling for multiple tests, one polymorphism, the rs1587097 *AVPR1A* polymorphism was significantly associated with vocal symptoms. In addition, the results indicated that the nominal effects of one *OXTR* polymorphism (rs2268493) on vocal symptoms might be partly mediated by cortisol.

Conclusion: A positive association between stress and vocal symptoms was observed in the sample of occupational voice users of elementary school teachers, as well as in the population based sample. Though the association between cortisol and vocal symptoms was relatively weak, the result is significant as cortisol is a biomarker of the physiological stress response and therefore provides clarity if individuals experience a stress response without having to rely on self-report. The results of the effects of *OXTR* and *AVPR1A* polymorphisms should be interpreted with caution. The specific association

between the receptor genes and oxytocin or vasopressin levels in the brain and in the body is not yet fully understood. The association between the receptor genes of these hormones and the glucocorticoid reactivity during stress is also still unclear. Analyzing genetic risk factors in communication science and disorders research is becoming more common and additional data on the potential relevance of cortisol and stress related genetic variants could help researchers design future studies.

ABBREVIATIONS

A	Adenine
AVP	Arginine vasopressin
AVPR	Arginine vasopressin receptor
AVPR1A	Arginine vasopressin receptor 1A gene
ANS	Autonomic Nervous System
C	Cytosine
CRH	Corticotropin-releasing hormone
DNA	Deoxyribonucleic acid
G	Guanine
GERD	Gastroesophageal reflux disease
GSA	Genetics of Sexuality and Aggression
HPA	Hypothalamic-pituitary-adrenocortical axis
LD	Linkage disequilibrium
LPRD	Laryngopharyngeal reflux disease
OXT	Oxytocin
OXTR	Oxytocin receptor
OXTR	Oxytocin receptor gene
PNS	Parasympathetic nervous systems
SAM	Sympathetic-adrenal medullary system
SLP	Speech Language Pathologist
SNP	Single nucleotide polymorphism
SNS	Sympathetic nervous system
T	Thymine
VEAW	Voice Ergonomic Assessment in Work Environment – Handbook and Checklist
VHI	Voice Handicap Index

1. INTRODUCTION

The voice is our most widely used tool for communication and good communication skills have become increasingly important during the last decades (Ruben, 2000). The poet Henry Wordsworth Longfellow describes the human voice as "the organ of the soul" and the description might, to some extent, explain and illuminate the fact that vocal symptoms and voice disorders influence quality of life (Cohen, Dupont & Courey, 2006; Merrill, Roy & Lowe, 2013; Murry, Medrado, Hogikyan & Aviv, 2004; Smith et al., 1996). Vocal impairment influences communication and can result in restriction of activity and participation (Ma, Yiu & Verdolini Abbott, 2007) as defined by the International Classification of Functioning, Disability and Health (ICF) (World Health Organization, 2001). The voice is an important tool in a variety of occupations and according to Vilkman (2004), approximately one third of the Finnish work force uses voice as an essential tool in their work. This also means that whether the voice works as it should, becomes an issue that not only influences well-being, but also employment and private and public economy (Ruben, 2000; Titze, Lemke & Montequin, 1997; Verdolini & Ramig, 2001).

The stress reaction is complex and the symptoms it causes are diverse. From a clinical perspective, the concept of stress as a risk factor for voice disorder is of importance, since some vocal symptoms and vocal complaints may be related to or occur secondary to stress (Giddens, Barron, Byrd-Craven, Clark & Winter, 2013). Treating only the symptom without fully investigating the cause, might not lead to lasting treatment outcomes (MacKenzie, Millar, Wilson, Sellars & Deary, 2001; Roy, Bless, Heisey & Ford, 1997).

Risk factors for vocal symptoms and voice disorders have been studied in regard to occupation (Fritzell, 1996; Titze et al., 1997, Williams, 2003) and especially teaching occupations (Martins, Pereira, Hidalgo & Tavares, 2014; Smith, Lemke, Taylor, Kirchner & Hoffman, 1998). In several studies where etiological factors for vocal symptoms have been investigated, stress has been present among participants (Giddens et al., 2013; Van Houtte, Claeys, Wuyts & van Lierde, 2012; Wellens & Van Opsal, 2008). Stress as a contributing factor

in specific voice diagnosis has also been investigated (i.e. Baker 2003; Dietrich, Verdolini Abbott, Gartner-Schmidt & Rosen, 2008). Even though many studies have identified stress as a common risk factor, few have yet studied the association between stress and voice in a way generalizable to the general population. Investigating the association in a population based sample could entail finding generalizable results regarding the role of acute or chronic stress in vocal symptoms. Though recent work (e.g. Dietrich & Verdolini Abbott, 2012; Helou, 2014) has brought the field forward, the nature and strength of the association between stress and occurrence of vocal symptom remain unclear. The present thesis investigates the association between stress and vocal symptoms with a focus on possible somatic stress markers, biological markers and potential genetic factors. Studying the biological perspectives could provide answers to current gaps in knowledge on the role of stress in vocal symptoms, possibly providing new evidence for theoretical frameworks and future study designs regarding vocal impairment.

1.1 Definitions of vocal symptoms

Before discussing the effects of stress on voice, it is of importance to define and describe some of the central terms and concepts used throughout the thesis.

There is a large variation in prevalence reports regarding vocal symptoms and disordered voice. In the Nordic countries, prevalence studies show that disordered voice or vocal symptoms occur among 13–17% of students (Linnasalo; 1990, Simberg, Sala & Rönnemaa, 2004) and 16% in the general population (Lyberg Åhlander, Rydell, Fredlund, Magnusson & Wilén, 2015). Studies from the US show a prevalence varying from 3–28% (Aronson, 1990; Roy, Merrill, Thibeault, Parsa, Gray & Smith, 2004; Roy, Merrill, Gray & Smith, 2005; Verdolini & Ramig, 2001). In Australia, the prevalence was measured during three time points (Russell, Oates & Greenwood, 2005) during adult life (7%); during the last year (4%) and at the time of the survey (3%), providing information regarding the consistency of prevalence of vocal complaints over time. The various results in the prevalence reports illustrate the importance of the definitions and operationalization used.

A generally-accepted statement is that a disordered voice is a result of changes in the function and/or structure of the laryngeal apparatus that prevent it from meeting the functional voicing needs of the speaker (Coyle, Weinrich & Stemple 2001; Stemple, Glaze & Gerdeman, 1996). In a study by Roy et al. (2004, p.283), voice disorder was considered to occur "any time the voice does not work, perform, or sound as it normally should, so that it interferes with communication". In a similar manner, Russell et al. (2005) defined voice problems as any difficulty that prevented the participant from doing all they wanted to with their voice. Linnasalo (1991, p. 25) operationalized a voice disorder by asking their participants "Do you have any problems with speaking, if yes, what kinds of problems?". Sala, Laine, Simberg, Pentti, and Suonpää (2001), and Simberg, Sala, Tuomainen, Sellman and Rönnemaa (2006), used a somewhat more stringent definition of dysphonia as two or more vocal symptoms occurring weekly or more often. This description was based on a screening questionnaire including six vocal symptoms, where participants reporting two or more vocal symptoms occurring weekly or more often, also had visible changes on their vocal folds (Sala et al., 2001) and deviant voice quality (Simberg & Sala, 2008).

Vocal symptoms is a term that is widely used in this thesis. The term "vocal symptoms" in contrast to "voice disorder" refers to symptoms in the throat, voice or vocal apparatus. *Voice disorder* occurs when an individual express concern about his or her voice being abnormal and not meeting the daily needs of the individual (American Speech-Language-Hearing Association [ASHA], 1993; Stemple, Glaze, & Klaben, 2010; Verdolini & Ramig, 2001). Additionally, vocal quality, pitch, and loudness might be inappropriate or differ in regard to the individual's age, gender, cultural background, or geographic location (Aronson & Bless, 2009; Boone, McFarlane, Von Berg & Zraik, 2010). Voice disorders are classified using a number of different systems; however, usually they are categorized as organic or functional (Verdolini, Rosen & Branski, 2006). The organic voice disorders are physiological in nature, resulting from a physical change in the voice mechanism or problems with the innervation to the larynx. In contrast, functional voice disorders result from insufficient or improper use of the vocal

mechanism, the physical structure being normal (Verdolini, Rosen & Branski, 2006).

To enquire generalizable results about the nature of the complex relationship between stress and voice, it might be preferable to study vocal symptoms, favorably in population based samples, instead of focusing solely on help seeking population or populations diagnosed with a specific voice disorder. Using a population based sample could allow a generalization of results into different contexts.

A voice demanding occupation is one of the environmental factors identified as a vocal risk factor (Vilkman, 2004; Williams, 2003). Persons in teaching occupations are often regarded as a specific risk group. In a study investigating voice patients in Sweden (Fritzell, 1996), individuals in teaching professions were the most common occupational group and they were overrepresented in relation to total number of teachers in the population at that time. Other occupational groups also experiencing frequent vocal symptoms are for example singers (Phyland, Oates & Greenwood, 1999), priests (Hagelberg & Simberg, 2015) and soccer coaches (Fellman & Simberg, 2016). Explanations to this lay in high vocal loading (Lyberg Åhlander, 2011), working conditions (Munier & Farrell, 2016) and other environmental risk factors that these groups are exposed to (Vilkman, 2004).

1.2 Stress

As used in common language, the term *stress* has a number of different meanings, and it is important to present some of the definitions employed. According to Tachè and Selye (1985, p. 5), stress is a "non-specific response of the body to any demand". Levi (1987) defines stress as interplay between demand, need and capacity. If the balance is disturbed, it may lead to negative cognitive, physiological and emotional processes that vary in duration and intensity. Lazarus and Folkman (1984) describe the stress process as the individual's abilities in coping and adaption. They focus on cognition and motivation, and states that the stress process amounts to an individual's way of handling internal and external demands depending on whether the situation is evaluated as strengthening or consuming of resources. Cohen,

Kessler & Underwood Gordon (1995, p.3) describe stress as a "process in which environmental demands tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place persons at risk for disease". This definition focuses on the association between stress and negative health outcomes and this is a common objective to study stress. There have been many approaches to defining stress, and I will present a concrete suggestion and provide more specific definitions shortly. *Allostasis* as defined by the Allostatic Load Model (McEwen, 1998), refers to physiological adaptive responses to a potentially stressful event. According to this definition, stress can be both beneficial and harmful. When the vital adaptive responses are activated and deactivated in a balanced way, the body is able to cope efficiently with environmental demands. *Allostatic Load* is the condition where the adaptive responses or allostatic systems are overstimulated, and stress becomes harmful. In this thesis, I will employ the definitions used in the Allostatic Load Model (McEwen, 1998).

In a more specific view of the concept, there are the *stressor*, the *stress response*, the *perception of stress* and *stress reactivity*. The stressor can be categorized by duration, type and setting (Dickerson & Kemeny, 2004; Dietrich & Verdolini Abbott, 2008). The stressor constitutes the physical, biological or psychological condition or experience that triggers the stress response. The stress response is the psychobiological and endocrine chain reaction that takes place in the body as a result of the stressor (Dickerson & Kemeny, 2004). The stress reactivity is modulated by genetic background as well as early experience and refers to the increase in physical or psychological activity the stressor causes in relation to baseline (de Kloet, Joëls & Holsboer, 2005).

Before describing the impact of stress on voice, I will present the basic concepts included in the stress reaction.

1.2.1 Concepts and pathways of stress

The advantage of the stress reaction is that the process increases the chances of survival in a changing internal and external environment using adaption (Lightman, 2008). The body has an ongoing regulation of the internal environment in the organism to uphold homeostasis, that is, physiological and mental balance (Logan & Barksdale, 2008). If the organism senses a

disruption to the homeostasis, it will lead to a compensatory reaction, which is the stress reaction (Goldstein & McEwen, 2002). As described in the Allostatic Load Model (McEwen, 1998), a rapid shut-down of the adaptive responses to stressors, is essential for recovery. If recovery does not occur in balance with the stressors, the adaptive responses are overstimulated (frequent stress or failed shutdown), are abnormal or fail to activate (lack of stress response due to exhaustion) (Lundberg, 2002). As a result of the repeated activation without time for recovery, there is an overexposure to stress hormones which over time leads to a dysregulation of the stress response.

1.2.1.1 Acute and chronic stress

The acute stress reaction has different pathways – the autonomic nervous system (ANS) and the neuroendocrine system. The ANS is connected to organs throughout the body and can react rapidly to change. It consists of the parasympathetic nervous system (PNS), responsible for rest and recovery, and sympathetic nervous systems (SNS), active during the so-called fight-or-flight response. The neuroendocrine responses involve the rapid sympathetic-adrenal medullary system (SAM) and the slower hypothalamic-pituitary-adrenocortical (HPA) axis (de Kloet et al., 2005; Lundberg, 2002). The SAM system results in the secretion of epinephrine and norepinephrine from the adrenal medulla. These hormones bring on a number of physiological changes; increased cardiovascular function, redistribution of blood flow from visceral organs to exercising muscles, more rapid breathing and a mobilization of energy to active muscles. The HPA axis is activated by the corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) released from the hypothalamus (Lightman, 2008). The CRH travels to the pituitary gland, triggering the release of adrenocorticotropic hormone (ACTH) into the blood stream, which results in a secretion of cortisol from the adrenal cortex. The cortisol travels back to the hypothalamus through the blood stream, where the secretion of circulating cortisol is regulated (Lundberg, 2002). If the amygdala is activated during the stress reaction, it stimulates the release of CRH and the HPA axis and SNS (Gold, 2015). Cortisol influences metabolism and has an anti-inflammatory effect during acute stress (Sapolsky, Romero, & Munck, 2000). In summary, the short term acute

stress leads to increased secretion of stress hormones, reduced pain sensitivity, increased blood coagulation, enhanced immune functions, reduced metabolic activity, and increased cognitive functions.

Clearly there are various stressors that could cause an acute stress response. Imbalance between threat and challenge, the feeling of not being in control of the situation, or negative social evaluation can constitute stressors (Kemeny, 2003). In a meta-analysis regarding various stressors, tasks involving both social-evaluative and uncontrollable elements were associated with the largest cortisol and adrenocorticotropic hormone changes and the longest recovery time compared to other stressors (Dickerson & Kemeny, 2004).

During chronic stress, the stress response and the HPA axis become dysregulated. This happens when the stress response is too frequent, fail to shut-down, the response system is oversensitive and eventually fail to activate due to exhaustion (McEwen, 1998). Exhaustion might thus be a symptom of chronic stress. The dysregulation of the HPA axis leads to chronically elevated cortisol levels that over time eventually result in attenuated cortisol levels (Lundberg, 2002). In contrast to acute stress, chronic or long-term stress is associated with increased pain sensitivity, elevated blood pressure, metabolic disturbance, impaired immune function and impaired memory function (Frodl & O'Keane, 2013).

1.3 The effects of stress on the voice

Since stress influences the body on a psychological, physical as well as immunological level, many vocal symptoms could be associated with the impact of stress (Dietrich & Verdolini Abbott, 2008; Giddens et al., 2013). Vocal symptoms that have been reported during or as a result of stress are unsteady voice, tension and strain in the vocal apparatus, vocal trembles or breaks, and problems with adjusting loudness and tempo (Smith & Seidel, 1982).

An association between stress and voice has been found using various study designs. Results from studies investigating the effect of acute stress on the voice have shown both acoustic and perceptual changes (review by Giddens et al., 2013; Mendoza & Carballo 1998; Mendoza & Carballo 1999; Schneider

et al., 2006; Tse, Wong, Whitehill, Ma & Masters, 2013; Van Lierde, Van Heule, De Ley, Mertens & Claeys, 2009). The individual differences of acoustic changes in the voice as a result of stress seem to be large.

In studies analyzing the relationship between stress and voice using self-report, a specific distinction between acute and long-term stress is rarely made. However, investigations regarding the link between stress and voice disorders or vocal symptoms, have been done in various populations such as high risk occupational voice users, help seeking populations and in the general population. Multiple studies have identified psychological or psycho-emotional factors as risk factors for voice disorders in the high-risk group of teachers (Chen, Chiang, Chung, Hsiao & Hsiao, 2010; Van Houtte et al., 2012; Kooijman et al. 2006; Sliwinska-Kowalska et al., 2006). Studies aiming at studying the more specific link between voice and stress related factors (for example anxiety, stress reactivity or coping) in occupational voice users, have found positive association between such factors and occurrence of vocal symptoms in professional singers (Kenny, Davis & Oates, 2004) and teachers (Gassull, Casanova, Botey & Amador, 2010; Giannini, Latorre Mdo, Fischer, Ghirardi & Ferreira, 2015; da Rocha & de Mattos Souza, 2013; Zambon Moreti & Behlau, 2014).

In help seeking populations, the effect of stress on voice has been studied in regard to different voice disorders. There are results connecting stress to vocal nodules (Goldman, Hargrave, Hillman, Holmberg & Gress, 1996), muscle tension disorders (Altman et al., 2005; Baker, Oates, Leeson, Woodford & Bond, 2014; Dietrich, Verdolini Abbott, Gartner-Schmidt & Rosen, 2008; Seifert & Kollbrunner, 2006; Van Houtte, Van Lierde & Claeys, 2010), patients with voice concerns (Misono et al., 2014), psychogenic disorders (Baker, 2002; Baker, 2003) and functional or non-organic voice disorders (Baker, Ben-Tovim, Butcher, Esterman & McLaughlin, 2012). Studies in healthy participants regarding the effect of acute stress on the vocal apparatus have provided further depth regarding the biological pathways involved (Dietrich & Verdolini Abbott, 2012; 2014; Helou Wang, Ashmore, Rosen, & Verdolini Abbott, 2013); for example, by showing role of the ANS and/or SAM on the laryngeal muscles and lower airways during acute stress.

A part from the differences in populations used in the research regarding psychosocial factors and stress affecting voice, the study designs vary, as well as the operationalization (reviews by Baker, 2008; Giddens et al., 2013). Furthermore, as mentioned by Baker (2008) and Oates & Winkworth (2008) the range and diversity of terminology regarding non-organic voice disorders or functional voice disorders also makes distinct conclusions and conclusive comparisons difficult to make. The hierarchy of evidence levels, according to the New JBI Levels of Evidence (Joanna Briggs Institute Levels of Evidence, 2013), ranges from the lowest level of evidence which derives from expert opinions and bench research (Level V) to the highest level of evidence obtained from systematic reviews of relevant randomized controlled trials (Level I). In the review of psychogenic and psychosocial factors in the development of functional voice disorders by Baker (2008), it was shown that the data lies in the two lowest levels of the evidence hierarchy.

Though the evidence confirming an association between stress and vocal symptoms seems relatively robust, the literature overview in this thesis show evidence only from the lowest levels of the evidence hierarchy. Furthermore, no study has yet investigated the association between stress and vocal symptoms in a large population based sample, which hinders the generalization of the results. Furthermore, there are still gaps in our knowledge about the biological underpinnings involved in the association between stress and vocal symptoms, especially regarding chronic stress.

1.3.1 Possible pathways for stress related vocal symptoms

The Psychobiological Framework of Voice Disorders by Dietrich and Verdolini Abbott (2008, p. 161), describes theoretical pathways of the relation between stress and laryngeal behavior and voice disorders. Together with the revised psychobiological framework by Helou (2014) (Figure 1), these two models provided a frame of reference for studying stress and its relationship to voice disorders. This framework proposes the engagement of the ANS, neuroendocrine responses (HPA and SAM system) and the somatic motor system in explaining the stress-voice relationship. Especially the role of the ANS and/or SAM on the laryngeal muscles (Dietrich, 2008; Dietrich &

Verdolini Abbott 2012; 2014; Helou et al., 2013) and lower airways (Helou, 2014) have been studied in relation to these frameworks.

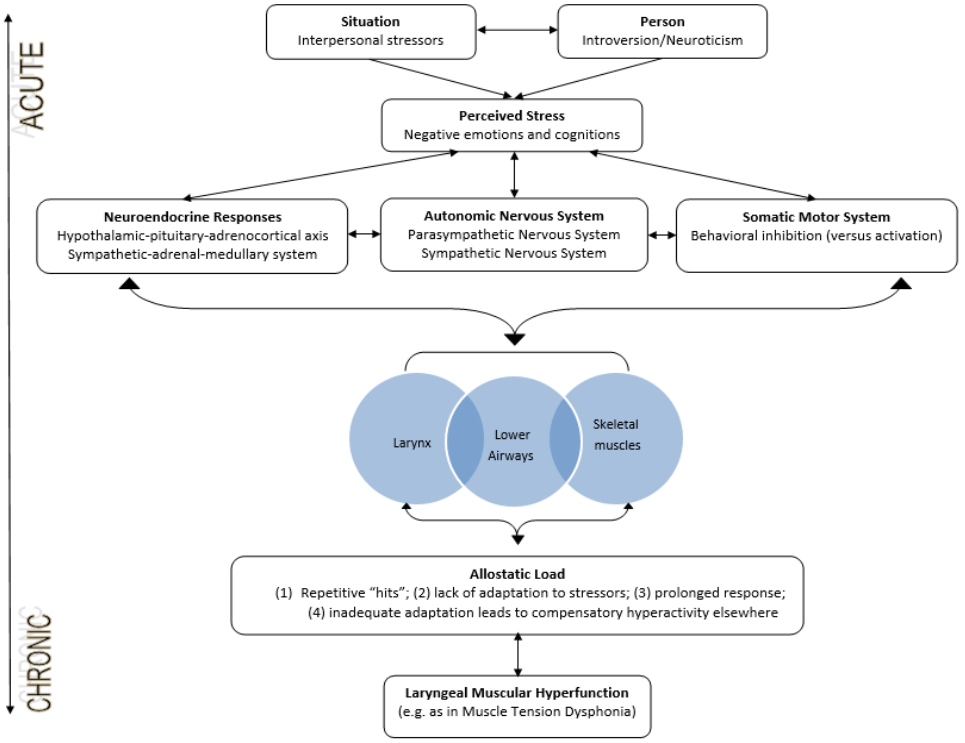

Figure 1. Revised Psychobiological Framework of Voice Disorders (Helou, 2014). Copyright by Dr. Leah Helou. Reprinted with permission.

Results from a number of studies show that general anxiety or tension is associated with increased muscle tension (Dahlström, Carlsson, Gale & Jansson, 1985; Flor, Birbaumer, Schute & Roos, 1991; Vantallie, 2002). For instance, temporomandibular dysfunction and the muscle hyper-function that this disorder involves are commonly associated with stress (Flor et al., 1991). The results of a study by Dahlström et al. (1985), showed a significant increase in masseter activity during stress. Insufficient rest of muscles may cause or maintain musculoskeletal disorders. According to the Cinderella hypothesis (Hägg, 1991), low threshold motor units may be kept continuously active due to psychological stress and as described by Lundberg (2002), an

overuse of these units may cause degeneration in the muscle cells or prevent repair in damaged muscle fibers. Since the laryngeal muscles, especially the extralaryngeal muscles, are very involved with the upper body physiology, these findings are informative in the study of stress and voice. There is also evidence that acute stress may increase intrinsic laryngeal muscles activity (Helou et al., 2013) and extralaryngeal muscle reactivity (Dietrich & Verdolini Abbott, 2014).

The stress response also increases the cardiovascular conduction. It involves vasoconstriction in non-active muscles and inner organs and redistribution of blood flow to activated muscles (Iversen, Iversen & Saper, 2000; Kemeny, 2003). The vascular supply to the larynx may thus be affected by psychological stress (Dietrich & Verdolini Abbott, 2008).

A result of the stress reaction is also that the secretion from the glands changes (Iversen et al., 2000). These changes might affect the larynx since numerous submucosal glands are located in the laryngeal and tracheal mucosa (Sato & Hirano, 1998). If changes in the mucosal secretion results in a reduction of secretion or a more viscous secretion, it might involve a decrease of local hydration in the vocal folds (Dietrich, 2008). There is an established negative relation between phonatory effort and hydration level (review by Hartley & Thibeault 2014; Sivasankar & Leydon; 2010). However, Sivasankar and Leydon (2010) conclude in their review that the role of the nervous system and hormones in mediating vocal fold hydration are still unclear.

Elevated cortisol levels due to long-term stress is associated with various symptoms, along with reduced immune function. Acute stress improves wound healing and resistance to infection (Dhabhar & McEwen, 1996) while chronic stress is immunosuppressive (Dhabhar, 2000). The negative impact of elevated cortisol levels on wound repair (Christian, Grahamf, Padgetta, Glasera & Kiecolt-Glaser, 2006; Gouin & Kiecolt-Glaser, 2011) has been confirmed regarding dermal wounds (Ebrecht, Hextall, Kirtley, Taylor, Dyson & Weinman, 2004) as well as mucosal wounds (Bosch, Engeland, Cacioppo & Marucha, 2007). The negative association between long term stress and wound healing is of relevance for voice production in regard to phonotraumatic lesions. The mechanism might be linked to proinflammatory

cytokines, which play an important role in the wound healing process, protecting the wound site from infection and preparing the wound for repair (Glaser, 2005, Glaser, Kiecolt-Glaser, Marucha, MacCallum, Laskowski & Malarkey, 1999). Higher levels of psychological stress has also been associated with greater symptom scores during upper respiratory infection (Cohen, Doyle, & Skoner, 1999). This could influence voice production if the level of stress or duration of stress leads to more often occurring upper respiratory infections, increased symptoms or delayed recovery.

Secondary symptoms of stress might possibly influence the vocal apparatus. One of these somatic symptoms associated with stress possibly affecting voice, is laryngopharyngeal reflux disease (review by Schneider, Vaezi & Francis, 2016). Secondary stress symptoms could co-occur with primary stress markers, such as increased laryngeal muscle tension or immunosuppressive changes due to chronic stress.

According to Coyle et al. (2001), the most common diagnose occurring in the treatment-seeking population with laryngeal pathologies is laryngopharyngeal reflux disease (LPRD). LPRD is caused by the direct effect of refluxed gastric contents on the upper respiratory tract, whereas gastroesophageal reflux (GERD) implies refluxed gastric contents mainly in the gastronomic tract (review by Rees & Belafsky, 2008). Though reflux of gastric contents is involved in both LPRD and GERD, the mechanisms and symptoms seem to be different in the two disorders (Gill et al., 2005; Rees & Belafsky, 2008). Whereas acid regurgitation and heartburn are the most frequent symptoms of GERD (Ronkainen et al., 2005), LPRD can occur without symptoms of heartburn (Rees & Belasky, 2008). In contrast to the esophagus, the mucosa in the larynx is not equipped to withstand gastric refluxate (Koufmann, 1991). Although the mechanisms causing LPRD are not entirely clear, they are hypothesized to be caused by laryngeal mucosa being repeatedly exposed to acid and pepsin resulting in a breakdown of defense mechanisms in the epithelium (Gill et al., 2005; Johnston et al., 2006). Thus, acid in the larynx and pharynx may lead to a number of mucosal changes (Khan, Hashmi, Elahi Tariq & Ingrams, 2006), possibly resulting in vocal symptoms such as sore throat, a feeling of a lump in the throat, chronic hoarseness, chronic cough and throat clearing (Hamdan, Sharara, Younes &

Fuleihan, 2001; Koufmann, 1991). As few as three episodes of laryngopharyngeal reflux can result in laryngeal damage, in case of a pre-existing mucosal injury (review by Rees & Belasky, 2008). There seems to be an association between severity of GERD and prevalence of LPRD, and the two conditions seem to be interlinked (Groome, Cotton, Borland, McLeod, Johnston, Dillon, 2007).

It is known that stress and emotions can affect the occurrence of gastric and intestine symptoms (Cassileht & Drossman, 1993; Drossman, 1996) and that psychosocial factors might be associated with the occurrence of GERD (Morrison, Rammage & Emami, 1999; Núñez-Rodríguez & Sivelo, 2008). Gastric distress and/or LPRD may in some cases be symptoms of stress. If the refluxed acid or pepsins reach past the upper esophageal sphincter, the reflux can have negative impact on the mucosa in the larynx and influence voice production.

However, as concluded in the review by Schneider et al. (2016); even though literature to date has shown a relationship between LPRD and vocal symptoms, the nature, strength and causality of that association remains unclear.

1.4 Gender differences

1.4.1 Gender difference in vocal symptoms

It is well established that voice disorders in adults are more common in women than in men (see e.g. Coyle, Weinrich, & Stemple, 2001; Herrington-Hall, Lee, Stemple, Niemi & McHone, 1988; Roy et al., 2005). Gender differences in voice disorders may partly have their explanations in anatomical differences (Titze, 1994; Titze, Hunter, & Svec, 2007) and possible differences in hyaluronic acid distribution in the vocal folds between the genders (Butler, Hammond, & Gray, 2001; Ward, Thibeault & Gray, 2002). Additional explanations may be that women use higher vocal effort (Södersten, Ternström, & Bohman, 2005) and that women are overrepresented in occupations with many voice ergonomic risk factors (Vilkman, 2004).

1.4.2 Gender differences in stress and cortisol levels

Gender differences also exist regarding the experience of stress (Cohen & Janicki-Deverts, 2012), cortisol secretion (Garvin, Hurwitz Eller & Harris, 2011; Swaab, Bao & Lucassen, 2005; Wüst et al., 2000b) as well as consequences of stress and stress coping (Lundberg & Cooper, 2011).

The stress response *per se* is universal and similar for men and women. The gender difference concerning stress is notable when it comes to health problems and their connection to stress and stress symptoms. In general, the studies concerning gender difference indicate that while women show more psychological reactions to stress (Mirowsky & Ross, 1995) and a higher tendency for depression (Piccinelli & Wilkinson, 2000), men show more physiological reactions to stress as well as negative behavioral patterns (Weekes, MacLean & Berger, 2005). Women and men have shown different patterns concerning biological function, behavior in seeking care as well as the perception of symptoms (Lundberg, 2008). The results of Weekes et al. (2005) showed that stress was a significant negative predictor of health for both men and women. However, the perception of stress was predictive only for women. Gender differences in stress responses seem to be present at the level of neuronal activation (Zavala, Fernandez & Gosselink, 2011) and may contribute to different consequences of especially chronic stress in men and women. Zavala et al. (2011) conclude that women may be more sensitive to glucocorticoid negative feedback, suggesting a gender difference in the efficiency in initiating and terminating the stress response.

With regards to cortisol as a marker for stress, Wüst et al. (2000b) found that gender significantly influenced early morning free cortisol levels, with women having higher levels than men. Similarly, in a review by Swaab et al. (2005) the results showed that women had higher cortisol levels than men. In a more recent review (Garvin et al., 2011), the authors concluded that there were no large differences between men and women with regard to cortisol levels, however, tendencies were found that women in general had somewhat higher levels than men but that men, regarding some stressors, had higher cortisol responses in laboratory stress test settings.

1.5 Genetic effects on voice

Studies on family history of voice disorders (Roy et al., 2004) as well as genetic syndromes with vocal pathologies (Van Borsel, 2004) have suggested the existence of a possible genetic predisposition to voice disorders. Comparing monozygotic and dizygotic twins enables researchers to disentangle the effects of genes and environmental effects from each other, and such twin studies have indicated that part of the variation between individuals in voice characteristics (Debruyne, Decoster, Van Gijsel &Vercammen, 2002; Van Lierde, Vinck, De Ley, Clement & Van Cauwenberge, 2005) as well as vocal symptoms (Simberg et al., 2009) are explained by genetic influences. The study by Simberg et al. (2009), showed that 35% of the variation in vocal symptoms between individuals was explained by genetic factors, confirming that an individual's genotype can predispose him or her to vocal symptoms. The rest of the variation was explained by non-shared environmental factors (i.e. factors making two twins in a pair different from each other). In addition, a genetic predisposition has been found regarding Reinke's edema (Thibeault, Gray, Li, Ford, Smith & Davis, 2002) and vocal fold polyp and granuloma (Thibeault, Hirschi & Gray, 2003). Findings of *FOXP2*-related speech and language disorders have included neuromuscular-based speech disorder, that may in turn affect resonance, voice quality and breath support during phonation (Morgan, Fisher, Scheffer, & Hildebrand, 2016; Shriberg et al., 2006).

Genetic effects have also been found regarding stress sensitivity (review by Bogdan, Nikolova & Pizzagalli, 2013), cortisol levels after awakening (Wüst, Federenko, Hellhammer & Kirschbaum, 2000a) and HPA-axis function (review by Frodl & O'Keane, 2013). Given the association between stress and voice symptoms, other candidate genes with potential effects on voice symptoms include genes related to stress and the stress response. Many of the genes that have been associated with the stress reaction or stress reactivity, are connected to the neuroendocrinology affecting the body and the brain during stress, including OXT and AVP. Variants in the oxytocin receptor gene (*OXTR*) (Love et al. 2012; Neumann & Landgraf, 2012),), and arginine vasopressin receptor 1A gene (*AVPR1A*) (Neumann & Landgraf, 2012) have been associated with various socio-behavioral and emotional domains (Auer,

Byrd-Craven, Grant, & Granger, 2015; Johansson et al., 2012a; Johansson, Westberg, Sandnabba, Jern, Salo & Santtila, 2012b; LoParo, Johansson, Walum, Westberg, Santtila, Waldman, 2015; LoParo & Waldman, 2014; Meyer-Lindenberg et al., 2011; Thompson, Parker, Hallmayer, Waugh & Gotlib, 2011; Walum et al., 2012; Walum et al., 2008; Westberg & Walum, 2015).

The specific association between stress and these receptor genes has been investigated to some extent, mainly regarding *OXTR*. Rodrigues, Saslow, Garcia, John and Keltner (2009) showed an association between the rs53576 polymorphism and cardiovascular reactivity across a variety of stressful contexts. Chen, Kumsta, von Dawans, Monakhov, Ebstein, and Heinrichs (2011) found that the same polymorphism was associated with benefit from social support during a psychosocial laboratory stress procedure measuring cortisol response to stress with and without social support. The rs53576 has also been associated with cortisol reactivity and rejection sensitivity (Auer et al., 2015). Myers et al. (2014), in turn, showed that the rs139832701 polymorphism was associated with mood and anxiety symptoms and history of early life stress. Love et al., (2012) found that genetic variants in the rs4813625 resulted differences in stress-induced dopamine release.

As described, previous studies on stress and voice have mainly focused on investigating and identifying environmental risk factors for voice symptoms largely omitting investigation of genetic risk factors. As described in Oates & Winkworth (2008) on the controversies in hyperfunctional voice disorders, there is considerable variability between individuals regarding the multifactorial nature of some voice disorders. The variability in current findings suggest a susceptibility or predisposition to vocal effects (Oates & Winkworth, 2008). The variance regarding complex phenotypes, such as vocal symptoms, is often explained by small effects from multiple genes, and though some genes have been indicated (such as the *FOXP2*) the vast majority are still unidentified. The variability between individuals needs to be thoroughly investigated in regard to genetic variants.

2 AIMS OF THE THESIS

The overall purpose of this thesis was to investigate the association between stress and vocal symptoms 1) in a sample of occupational voice users and 2) in a population based sample. Regarding the population based sample, the aim was to study this association using a biological stress marker, and by exploring whether possible genetic risk factors associated with stress would explain also variation in vocal symptoms between individuals, given the association between stress and vocal symptoms.

Study I

The aim of the first study was to investigate the connection between stress and vocal symptoms in a sample of occupational voice users at high risk for vocal symptoms; namely teachers.

Study II

The aim of the second study was to investigate whether there is a significant association between vocal symptoms and four possible phenotypical indicators of stress in a population based sample of twins and their siblings; feeling exhausted, feeling nervous or tense in situations where one is required to talk, experiencing heartburn and having been diagnosed with reflux disease. Additionally, we aimed at investigating possible gender differences in the occurrence of the stress indicators and vocal symptoms.

Study III

The aim of the third study was to investigate whether participants who reported more often occurring vocal symptoms showed higher salivary cortisol levels. The participants in this study were a subset of the same population based sample as in Study III. We wanted to investigate if a possible association between stress and vocal symptoms could be found when using a biological stress marker.

Study IV

As shown by Simberg et al. (2009), part of the variation in vocal symptoms is due to genetic influences, the aim of this explorative study was to investigate whether polymorphisms in the *OXTR* and *AVPR1A* genes are associated with

the occurrence of vocal symptoms. These receptor genes have been associated with social behavior and stress. In addition, we wanted to explore whether potential associations between polymorphisms of these genes and vocal symptoms could be partly mediated by cortisol given the involvement of both cortisol, oxytocin and vasopressin in the psychoendocrinology of the stress reaction.

3 METHODS AND MATERIAL

3.1 Participants

The results of the present thesis were based on two different projects. Study I was part of the project *Acoustics and voice ergonomics in classrooms – from investigation to intervention* (Opetustilojen akustiikka ja ääniergonomia - tutkimuksesta toteutukseen). The aim of the this project was to investigate the connection between voice ergonomic risk factors as well as room acoustics in elementary school classrooms and the teachers' voice related problems, stress levels and noise obtrusiveness (Työsuojelurahasto, 2012). Studies II, III and IV were part of the project *Genetics of Sexuality and Aggression* (GSA), investigating genetic and environmental effects on a range of phenotypes (Johansson, 2013), including voice-related ones for a subsample (Simberg 2009). The GSA-project is based on questionnaire data from population-based samples of twins and their siblings. In addition, hormone and DNA samples have been collected for a subsample.

The participants in Study I consisted of 39 Finnish elementary school teachers of which 31 were women (mean age 45 years, range 27–47 years) and 8 were men (mean age 39 years, range 31–45 years). A total of 39 classrooms in 14 Finnish elementary schools located in five different municipalities were assessed in the study. The participants were recruited via the head teachers of the schools, who at random selected the participating teachers and their classrooms for the study.

In Study II, a cohort of 1728 participants (555 men, 1173 women) completed a questionnaire concerning speech, language and voice, designed for the GSA project. The sample is based on two different GSA data collections. The first

data collection targeted Finnish twins born 1961–1971. The second data collection targeted Finnish twins born 1972–1989. From the first data collection, a total of 1289 twins received the speech, language and voice questionnaire of which 684 (53%) completed the questionnaire. In the second data collection, participants were invited to respond to the speech language and voice questionnaire after having completed a questionnaire addressing sexuality and aggression that was sent to 5026 twins. A total of 1044 (21%) completed the speech, language and voice questionnaire. In addition to completing the questionnaire, the participants from the second data collection were also asked to give a saliva sample for DNA and hormone analysis. A total of 657 participants (219 men, 438 women) of those who had also responded to the speech, language and voice questionnaire provided saliva samples for DNA analysis and 170 participants provided saliva samples for hormone analysis (49 men, 121 women). Study II includes all participants from the GSA sample that had responded to the speech, language and voice questionnaire, whereas the samples used in studies III and IV are subsamples based on different inclusion criteria used (see Table 1). Additional information regarding the four original studies is presented in Table 1.

Table 1. Summary of sample characteristics, research questions and measures used in the four original studies.

Study	Main study queries	Sample	Participants (*n*)	Main instruments/ Material	Method	Inclusion criteria
I	Phenotypic associations between voice ergonomic risk factors, vocal symptoms and VHI	Elementary school teachers	39 8 men 31 women	VEAW VHI Voice questionnaire	Survey data	
II	Association between possible stress markers and vocal symptoms	GSA: Population based	1728 555 men 1173 women	SCREEN6	Survey data	
III	The effect of cortisol on vocal symptoms	GSA: Population based	170 49 men 121 women	SCREEN6 Cortisol levels	Survey data Hormonal association study	Returned hormone samples with successful cortisol analysis
IV	The association between *OXTR* and *AVPR1A* SNPs and vocal symptoms	GSA: Population based	657 219 men 438 women	SCREEN6 Genotype data	Survey data Molecular-genetic association study	Returned DNA sample which was successfully genotyped

GSA = Genetics of Sexuality and Aggression, SNP = Single Nucleotide Polymorphism, *OXTR* = Oxytocin receptor gene, *AVPR1A* = Arginine vasopressin receptor 1A gene, VHI = Voice Handicap Index, VEAW = Voice Ergonomic Assessment in Work Environment – Handbook and Checklist, SCREEN6 = A six-item voice symptom questionnaire (Ohlsson, Andersson, Södersten, Simberg, & Barregård, 2012; Sala et al. 2001; Simberg & Sala, 2008).

3.2 Measurement of vocal symptoms (Studies I, II, III, IV)

In Study I, two sets of questions were used to measure vocal symptoms. The first set consisted of the following seven items; *Voice tires easily*, *Hoarseness*, *Voice breaks*, *Aphonia lasting at least a couple of minutes during speaking*, *Difficulty in being heard*, *Throat clearing* and *Sore throat or globus in the throat*. The

participants reported how often these vocal symptoms had occurred during the last 12 months on a five-point scale; 4 = daily or almost daily, 3 = weekly or almost weekly, 2 = monthly or almost monthly, 1 = more seldom, 0 = no symptom (Cronbach's α = .89). The questionnaire was formed based on the Tuohilampi Questionnaire, which is a pool of questions and question sets for epidemiologic studies designed for epidemiological studies of environmental or work-related symptoms or diseases of respiratory organs, skin and eyes in the adult population (Susitaival & Husman, 1997). The question sets in the Tuohilampi Questionnaire are designed to make national and international results more comparable and are based on recommendations from the World Health Organization (WHO) and the American Thoracic Society (ATS).

Additionally, we used the Finnish-validated version of the Voice Handicap Index (VHI) (Alaluusua & Johannson, 2003; Jacobson et al., 1997) to measure vocal symptoms and of the consequences of a voice disorder in daily. The VHI consists of 30 items divided into three different domains; physical (VHI-P), functional (VHI-F) and emotional (VHI-E). The VHI uses a five-point scale; 4 = always, 3 = almost always, 2 = sometimes, 1= almost never, 0 = never, with a maximum of 40 points for every domain and 120-point total (Cronbach's α = .94).

In Studies II-IV a version of the voice symptom questionnaire referred to as SCREEN6 (Ohlsson, Andersson, Södersten, Simberg, & Barregård, 2012) was used. The questionnaire included the following vocal symptoms; *Voice becomes strained or tired*, *Voice becomes hoarse or low in pitch*, *Voice breaks while talking*, *Difficulty in being heard*, *Throat clearing or coughing while talking* and *Sensation of muscle tension or a lump in the throat*. The participants reported how often these vocal symptoms had occurred during the last 12 months on a four-point scale; 3 = daily, 2 = weekly, 1 = more seldom, 0 = never.

The main difference between the voice questionnaire used in Study I and the SCREEN6, was that the questionnaire in Study I included a seventh item (*Aphonia lasting at least a couple of minutes during speaking*). Also, the item *Sensation of muscle tension or a lump in the throat* used in the version of SCREEN6, did not include the concept of sore throat, which was included in the questionnaire used in Study I. Apart from these differences the

questionnaires were very similar and both have been widely used (Nybacka, Simberg, Santtila, Sala & Sandnabba, 2012; Sala et al., 2001; Simberg, Sala & Rönnemaa, 2004; Simberg et al., 2006; Simberg et al., 2009). In a study by Sala et al. 2001 the questions used in Study II-IV, were compared with examination performed by a laryngologist regarding organic changes on the vocal folds. Participants who reported two or more vocal symptoms occurring weekly or daily, often had visible organic changes on their vocal folds (Sala et al. 2001) and deviant voice quality (Simberg & Sala, 2008).

A composite variable of the vocal symptoms in SCREEN6 was used in Studies III and IV. The composite variable was calculated based on the sum of the vocal symptoms with higher values indicating more vocal symptoms (range 0-18). Since a principle component analysis is more reliable when conducted on a larger sample, the composite variable was based on an analysis conducted on the same voice-variable set as in Studies III and IV, but with 1728 participants (Simberg et al., 2009). The sample in Simberg et al. (2009) is the same sample as used in Study II (N = 1728) and an overlapping sample of Study III (N = 170) and IV (N = 657). Using the analysis of Simberg et al. (2009) rendered a more reliable result than if conducting an analysis based on the samples in Studies III or IV. Principal components was used as extraction method. All variables had high and significant inter-correlations and the Kaiser-Meyer-Olkin measure of sampling adequacy was .85 with Barlett's test of sphericity also being significant (p < .001), which suggested that the items could be subjected to a principal components analysis. Both the eigenvalues and the visual examination of the scree plot suggested that a one factor solution would be acceptable, explaining 57% of the variance. Component loadings ranged 0.69 – 0.80 and the resulting scale was reliable (Cronbach's α = .84) (Simberg et al., 2009).

3.3 Measurement of stress (Studies I, II, III) and voice ergonomic risk factors (Study I)

The Voice Ergonomic Assessment in Work Environment – Handbook and Checklist (VEAW) (Sala et al., 2009) was used for assessing voice ergonomic risk factors in Study I. The assessment was carried out at the end of the teacher's workday. The data were collected by measuring, observing or

asking the teacher questions. The VEAW consists of six domains or risk fields; *working culture*, *noise*, *indoor air quality*, *working posture*, *stress* and *access to a sound amplifier as an aid to voice production*.

All risk fields except *stress* and *access to a sound amplifier* included multiple risk factors. If the conditions for a factor were in line with the recommendation described in the VEAW the factor was scored as zero (0 = carried no risk for the voice). If the conditions were not in line with the recommendation the factor was scored as one (1 = risk for voice disorder). The domain stress was rated on a five-point scale (0 = no stress, 1 = only a little, 2 = some, 3 = quite a lot, or 4 = very much stress). Here the rating 3 and 4 were considered a risk for voice disorder. The risk factors and their scoring are presented in the Appendix of the original study (Study I).

Study I is the first study where the VEAW has been used. During the data collection with the assessment material a reliability analysis was computed, were two of the researchers in the research group assessed eight classrooms separately (Cronbach's α was .99 for the total number of the risk factors and ranged from .95 to 1 for the different risk fields).

The questionnaire concerning speech, language and voice, designed for the GSA project (Studies II and III), included questions that could be regarded as potential symptoms of stress or symptoms affecting voice that may be secondary to stress. The following four items were chosen for Study II; *Do you feel strained or exhausted*, *Do you feel nervous or tense in situations where you are required to talk*, *Have you been diagnosed with reflux disease* and *Do you suffer from heartburn*. Participants could answer "yes" or "no" to these questions. The questionnaire was formed based on the guidelines of the Tuohilampi Questionnaire (Susitaival & Husman, 1997). In Study III the item *Do you feel strained or exhausted* was also used as a covariate in order to analyze whether levels of cortisol explained interindividual variation in vocal symptoms not captured by self-reported exhaustion.

3.4 Hormone analysis and genotyping

Cortisol levels were extracted from samples of saliva for studies III and IV. The Salivette® (SARSTEDT AG & Co., Nümbrecht, Germany) hormone

sampling kit was used when collecting saliva from participants. Participants were advised to provide saliva samples in two collection tubes, in the morning after waking up, preferably before 9 a.m. They were also advised not to eat, drink, brush their teeth, or take any medication prior to giving the samples. Altogether 86.5% of the participants reported having given the saliva sample before 9 a.m. (*M* = 7:50 a.m., *SD* = 86 min). The extraction of cortisol levels was carried out at the Sahlgrenska University Hospital, Clinical Chemistry, Gothenburg, Sweden. The cortisol levels used in Study III ranged from 1.7 to 104.76 nmol/L. According to the Mayo Clinical laboratory in the United Stated the reference values for morning cortisol are 2.8–20.7 nmol/L. These are the reference values used in Finland by the laboratory of the Helsinki University Central Hospital HUSLAB.

DNA extracted from saliva samples was used in Study IV. These saliva samples were collected from participants using the Oragene™ DNA self-collection kits (DNA, Genotek, Inc., Kanata, Ontario, Canada). The genotyping of SNPs was performed by KBioscience in the UK (www.lgcgenomics.com) using the KASPar chemistry, which is a competitive allele specific polymerase chain reaction SNP genotyping system performed with Fluorescent Resonance Energy Transfer quencher cassette oligos (http://www.lgcgroup.com/products/kasp-genotyping-chemistry/#.Vs8ALtBFoUM). The distribution of genotypes did not deviate from Hardy–Weinberg equilibrium (Hartl & Clarke, 2007).

In Study IV a total of thirteen *OXTR* and six *AVPR1A* SNPs were genotyped and analyzed. The analyzed polymorhisms and their distributions are shown in Table 2.

Table 2. The 19 analyzed oxytocin receptor gene (*OXTR*) and arginine vasopressin 1A receptor gene (*AVPR1A*) single nucleotide polymorphisms (*N* = 657[a]), their nucleotide compositions and genotype distributions.

rs number	SNP/ nucleotide combination	Common homozygotes (*n*)	Heterozygotes (*n*)	Rare homozygotes (*n*)
OXTR				
rs75775*	G/T	G: 233	147	30
rs1488467*	C/G	G:381	28	1
rs4564970*	C/G	G: 372	36	1
rs4686302*	C/T	C: 299	99	9
rs237897	A/G	G:104	211	92
rs53576	A/G	G: 131	211	65
rs2254298*	G/A	G: 354	52	4
rs2268493	C/T	T:151	203	54
rs237887	A/G	A:122	206	85
rs1042778	G/T	G: 181	167	62
rs7632287*	A/G	G: 212	164	32
rs11720238*	G/T	G: 333	69	8
rs2270465*	C/G	G:213	158	36
AVPR1A				
rs10877970*	C/T	T:297	103	8
rs10877969*	C/T	T: 293	98	7
rs3021529*	A/G	G: 333	69	5
rs1042615	A/G	G: 142	196	75
rs11174811*	A/C	C: 330	70	5
rs1587097*	T/C	C: 362	46	3

SNP, single nucleotide polymorphism; G, guanine; T, thymine; C, cytosine; A, adenine.

*The rare homozygotes were grouped together with the heterozygotes for the analyses.

[a] The *N* per row may not always add up to *N* = 657, since some SNPs were not identified for all individuals.

3.5 Statistical analyses and study design

The analyses in Study I were carried out using PASW Statistics 18.0 software for Windows/Mac operating system (SPSS, Inc., Chicago, IL). The connection between the voice ergonomic risk factors, vocal symptoms and VHI were analyzed using the Spearman rank correlation coefficient.

For Studies II-IV the Statistical Package of Social Sciences (SPSS) for Windows was used (vers. 19 Study II; vers. 21 Studies III and IV). In studies II, III and IV we used the Generalized Estimated Equations (GEE). The GEE is an extension to the Generalized Linear Model to data with an unknown correlation structure between the measurements and this method takes into account the dependent structure of family data. Observations from members of the same family can be clustered due to genetic or environmental influences shared between family members. Choosing a method taking into account such potential inter-dependence between subjects, made it possible to include all siblings and twins from a family in the analysis (Hanley, Negassa, Edwardes, & Forrester, 2003). The GEE together with the robust variance estimator is fairly robust in yielding consistent and asymptotically normally distributed parameter estimates even in cases in which the working correlation matrix is misspecified (Gardiner, Luo & Roman, 2009).

In study II, the relationship between possible stress markers and vocal symptoms were analyzed using the GEE with an ordinal response model. This method was also used to determine a possible gender difference regarding the association between stress markers and vocal symptoms. The correlation between the dichotomous stress markers was calculated using a phi-correlation.

In study III winsorization was used for the cortisol values to reduce the effect of potentially spurious outliers by setting outliers to 3 *SD* from the mean. Since vocal symptoms were more frequent among women and cortisol levels were higher for women, gender was added as covariate in the GEE analysis as well as an interaction between gender and cortisol. In addition, we reported Pearson's correlations between the vocal symptoms and level of cortisol as a way of depicting estimates of effect sizes to ease interpretation of the results of the GEE analysis. Persons with anxiety or depression might have lower

threshold for experiencing somatic symptoms in general possibly resulting in a higher prevalence of reported health complaints in this population (Haug, Mykletun & Dahl, 2004; Katon, Lin, Von Korff, Russo, Lipscomb & Bush, 1991; Nokao, Yamanaka & Kubecki, 2001). To control for an overall tendency to report health complaints, symptoms of anxiety and depression (as measured using the Brief Symptom Inventory; Derogatis, 2001) were included in Study III.

In study IV we tested genotype effects of thirteen *OXTR* and six *AVPR1A* SNPs on the occurrence of vocal symptoms, again using GEE. Gender was included as a covariant in all analyses. Multiple testing was corrected for by changing the significance threshold required to keep Type 1 error rate at 5% to $p = .00341$. This was done according to the method by Nyholt (2004), using an estimate of effective number of independent variables proposed by Li and Ji (2005). This method takes into account linkage disequilibrium (LD) between polymorphisms, that is, that all polymorphisms are not independently inherited. Gene-based effects were also tested for regarding the *OXTR* and the *AVPR1A* genes, respectively, on the occurrence of vocal symptoms. This was done using the versatile gene-based test for genome-wide association studies (VEGAS) (Liu et al., 2010). Since two genes were tested for association, a Bonferroni corrected *p*-value of .025 was used to indicate a significant association in the gene-based tests after correcting for multiple tests.

To analyze whether part of the associations between the SNP and vocal symptoms could be mediated through cortisol, the SNPs with nominal effects on vocal symptoms (i.e. $p < .05$) were chosen for further analyses of such mediational effects. This was done using the three first steps in the mediational model described by Kenny and Judd (2014). The steps consist of showing that the independent variable (SNP) is associated with the outcome variable (vocal symptoms) (step 1), that the independent variable is associated with the mediator (cortisol) (step 2), and that there is an association between the mediator (cortisol) and the outcome (vocal symptoms) when controlling for the independent variable (step 3).

4 RESULTS

4.1 Descriptive statistics of phenotypic data and gender differences

The occurrence of vocal symptoms among the teachers (sample used in Study I) is presented in Table 3. The most common vocal symptom among the participants in Study I was that the voice tires easily. The VHI-total score showed a mean of 18 (median 17, range 1–58, where the maximum possible score is 120 points). Of the subscales, the physical domain showed the highest scores compared to the functional and emotional domains.

Participants in Studies II-IV were all part of the population-based GSA sample. In studies II-III the most common vocal symptom was need of throat clearing or coughing while talking. The occurrence of vocal symptoms in the GSA-sample are also presented in Table 3. Vocal symptoms occurring at least weakly were more commonly reported in the sample of teachers than the population based sample for three out of five vocal symptoms that were measured in both samples (Table 3).

Table 3. Vocal symptom occurring at least weakly as reported in the original studies.

	Sample of teachers (n = 39)		GSA-sample (n = 1728)		Fisher's exact test
Symptom	%	n	%	n	p
Voice tires easily	54	21	11.1	191	<.001
Hoarseness	36	14	12.5	216	<.001
Voice breaks	13	5	7.5	130	.22
Aphonia	3	1	NA	NA	NA
Difficulty in being heard	15	6	9.8	168	.27
Throat clearing	41	16	25.5	441	.04
Sore throat or globus	31	12	NA	NA	NA
Sensation of muscle tension or a lump in the throat	NA	NA	14.2	246	NA

NA = information not available. GSA = Genetics of Sexuality and Aggression.
Note. GSA-sample as measured in Study II in the complete sample (as opposed to the subsamples used in studies III and IV).

Table 4. Descriptive data regarding the stress related findings in Studies I-IV

Stress related variables	Sample of teachers (n = 39)		GSA-sample (n = 1728)	
			Yes	
	%	*N*	%	*n*
Stress*	28.2	11		
Feeling nervous or tense in situations where you are required to talk			45.3	783
Suffer from heartburn			39.4	526
Feeling strained or exhausted			26.9	464
Being diagnosed with reflux disease			4.0	69

GSA = Genetics of Sexuality and Aggression

* The domain stress was rated 0–4 (0 = no stress, 1 = only a little, 2 = some, 3 = quite a lot, or 4 = very much stress). Individuals were rated as stressed if they had reported 3 or 4.

Note. GSA-sample as measured in Study II using the complete sample (as opposed to the subsamples used in studies III and IV,) dichotomous variables (yes/no).

The descriptive data regarding the stress related findings in Studies I–IV are presented in Table 4. Voice ergonomic risk factors were found in all 39 classrooms in Study I. Stress was a risk factor for 28.2 % of the teachers in Study I. Similar levels of stress were indicated in the GSA-sample, as measured in Study II in the complete sample, where exhaustion was reported among 26.9 % of the participants. Regarding the other stress markers used in study II the most common was feeling nervous or tense in situations where you are required to talk, which was reported among 45.3% of the participants. A total of 39.4% suffered from heartburn and 4% had been diagnosed with reflux disease.

Gender differences in levels of vocal symptoms and stress were analyzed in the GSA-sample. All six vocal symptoms were significantly more common among women, as analyzed in Studies II and III. Regarding the stress variables, exhaustion was significantly more common among women. Women also reported feeling nervous or tense in situations where they are required to talk significantly more often than men. The gender differences regarding vocal symptoms and stress markers are shown in Table 5.

Exhaustion showed a positive correlation with the other three stress markers. Both for men and women exhaustion showed a positive correlation with being nervous or tense in situations that require talking (ϕ men = 0.152, $p < .05$; ϕ women = 0.143 $p = .01$) and heartburn (ϕ men = 0.112, $p < .05$; ϕ women = 0.091, $p = .01$). The correlations between exhaustion and diagnosed reflux disease was significant only for men ($\phi = 0.104$, $p = .05$). In study III, women had higher salivary cortisol levels than men, with the difference showing tendency towards statistical significance.

Table 5. Gender differences regarding vocal symptoms and stress markers (Study II GSA sample, $n = 1728$)

Vocal symptom/	Men Σ Weekly and Daily (%)	Women Σ Weekly and Daily (%)	*Wald* χ^{2} [a]
Voice becomes strained or tired	4.0	14.4	44.06***
Voice becomes hoarse or low in pitch	6.2	15.5	19.62***
Voice breaks while talking	4.7	8.9	13.68***
Difficulty in being heard	7.9 [b]	10.6 [b]	4.77*
Throat clearing or coughing while talking	21.1	27.9	7.62**
Sensation of muscle tension or a lump in the throat	9.4	16.7	7.03**
Stress marker	Men Yes (%)	Women Yes (%)	
Feeling nervous or tense in situations where you are required to talk	43.6	46.1	17.528***
Being diagnosed with reflux disease	4.0	4.0	0.002
Suffer from heartburn	30.3	30.5	0.000
Feeling strained or exhausted	22.3	29.0	48.415***

* $p < .05$; ** $p < 0.01$; *** $p < 0.001$. [a] $df = 1$. [b] Discrepancies in values with Study II is due to errors in the original study.

4.2 Associations between stress and vocal symptoms (Studies I-III)

Of all the risk factors in Study I, the strongest positive correlation was found between stress and the physical domain of the Voice Handicap Index (VHI-P). Stress was the only risk factor that also showed a significant positive association with both the total VHI-score and the vocal symptoms measured with the seven-item voice questionnaire. As shown in Table 6, the correlation coefficient was quite similar for all the voice ergonomic risk fields.

Table 6. Significant correlations between voice ergonomic risk factors and vocal symptoms, $N = 39$.

Voice ergonomic risk factors	7-item voice questionnaire Spearman's *r*	VHI (Total Score) Spearman's *r*
Working culture		0.34*
Noise		0.29*
Indoor air quality	0.28*	
Working postures	0.32*	
Stress	0.31*	0.30*
Sound amplifier as an aid		
All fields	0.41*	0.36*

* $p < .05$, VHI = Voice Handicap Index

Three out of the four possible stress markers in Study II showed a positive association with all six vocal symptoms. The occurrence of reflux disease was the only stress marker positively associated with only some of the vocal symptoms; *Voice becomes strained or tired*, *Voice becomes hoarse or low in pitch and Sensation of muscle tension or lump in the throat*. The vocal symptom *Sensation of muscle tension or lump in the throat* was the only symptom that was associated with all four stress related variables.

4.3 Association between cortisol and vocal symptoms

Cortisol levels, a widely-used biomarker for stress, showed a significant positive association with the composite variable of vocal symptoms (Study III). When the vocal symptoms were analyzed separately the positive association was significant for the following vocal symptoms; *Difficulty in being heard*, *Throat clearing or coughing while talking* and *Sensation of muscle tension or a lump in the throat*. The association between cortisol and vocal symptoms was similar for both men and women as indicated by non-significant interactions between gender and cortisol on voice symptoms.

Since stress is a risk factor for vocal symptoms, as also indicated by the association between exhaustion and the composite variable of vocal symptoms (Study II), the exhaustion variable was included in the analysis as a covariate to analyze whether cortisol would have an effect on vocal symptoms, independent of variation captured by the exhaustion variable. The results showed that the magnitude of the effect of cortisol (as indicated by its unstandardized regression coefficient) on vocal symptoms was somewhat lower when exhaustion was included as covariate, however, the confidence intervals for the effect of cortisol on vocal symptoms were overlapping between the two models. The effect of cortisol was no longer significant when controlling for the effect of exhaustion, however the lack of significance was likely affected by lower statistical power due to missing values for the exhaustion variable and therefore a smaller sample size (n = 112).

The association between exhaustion and cortisol was not significant. The association between cortisol and vocal symptoms remained significant (*Wald* χ^2 = 6.628, df = 1, p = .01, B = 0.049, SE = 0.019) after controlling for symptoms of anxiety and depression (as measured using the Brief Symptom Inventory; Derogatis, 2001). This suggested that the results seem specific to vocal symptoms instead of an overall tendency to report health complaints.

4.4 Associations between *OXTR*, *AVPR1A* and vocal symptoms

As shown in Table 7, three *OXTR* polymorphisms (rs2270465, rs2268493, rs7632287) and two *AVPR1A* polymorphisms (rs1587097, rs1042615) showed

nominally significant effects on vocal symptoms (i.e. significant effects before correction of *p*-value threshold due to multiple testing).

Regarding the rs7632287 SNP, carriers of one or two copies of the adenine (A) allele (A:A/ A:G), showed more often occurring vocal symptoms (M = 4.93, SE = 0.24) than homozygous participants for the guanine (G) allele (G:G) (M = 4.01, SE = 0.22). Participants homozygous for the cytosine (C) allele (C:C) on the rs1587097 showed more often occurring vocal symptoms (M = 4.60, SE = 0.18) than participants who were carriers of one or two copies of the thymine (T) allele (T:T/ T:C) (M = 3.31, SE = 0.40). Participants homozygous for the adenine (A) allele (A:A) on the rs1042615 showed more often occurring vocal symptoms (M = 5.35, SE = 0.42) than participants who were homozygous for the guanine (G) allele (G:G) (M = 4.36, SE = 0.28) or heterozygous (A:G) (M = 4.17, SE = 0.23).

After the α-level was corrected to account for multiple testing (α = 0.00341) the effect of one of the *AVPR1A* SNPs, the rs1587097, remained significant (*Wald* χ^2 = 8.847, p = .0029). Figure 2 shows the association between the rs1587097 polymorphism and the occurrence of self-reported vocal symptoms.

Table 7. The effects of the oxytocin receptor gene SNPs and the arginine vasopressin 1A receptor SNPs on vocal symptoms.

SNP	*Wald* $\chi 2$	*df*	*p*
OXTR			
rs75775*	1.975	1	0.160
rs1488467*	0.666	1	0.414
rs4564970*	0.073	1	0.787
rs4686302*	0.114	1	0.736
rs237897	1.476	2	0.478
rs53576	0.066		0.968
rs2254298*	0.651	1	0.420
rs2268493	6.246	2	0.044
rs237887	0.077	2	0.962
rs1042778	0.831	2	0.660
rs7632287*	7.842	1	0.005
rs11720238*	1.485	1	0.223
rs2270465*	5.412	1	0.020
AVPR1A			
rs10877970*	0.003	1	0.959
rs10877969*	0.014	1	0.904
rs3021529*	1.31	1	0.252
rs1042615	6.201	2	0.045
rs11174811*	1.1	1	0.294
rs1587097*	8.847	1	0.003

SNP = Single Nucleotide Polymorphism, *OXTR* = Oxytocin receptor gene, *AVPR1A* = Arginine vasopressin receptor 1A gene, *The rare homozygotes were grouped together with the heterozygotes.

Note. Gender was included as a covariate. Multiple testing was corrected for by changing the significance threshold required to keep Type 1 error rate at 5% to $p = .00341$.

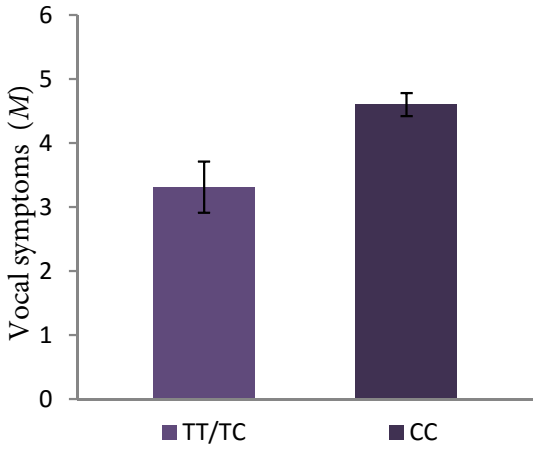

Figure 2. Association between *AVPR1A* rs1587097 polymorphism and the occurrence of self-reported vocal symptoms during the past 12 months (daily = 3, weekly = 2, less frequent = 1 or never = 0, composite variable range 0 - 18). T, thymine; C, cytosine.

The gene-based association analysis showed nominal tendencies for effect of the *OXTR* gene (p = .095) and the *AVPR1A* gene (p = .079) on vocal symptoms. These effects did not, however, pass the gene-based significance threshold corrected for multiple testing (set at p = .025).

The five polymorphisms with nominally significant effects on vocal symptoms were chosen for further analysis of mediational effects by cortisol. For mediation to occur, these SNPs would have to show effects on cortisol (second criterion for mediation). Only one SNP (*OXTR* rs2268493) showed a significant effect on cortisol levels (*Wald* χ^2= 8.346, p = 0.015), and therefore, only this SNP was analyzed further for possible mediation. Participants homozygous for the cytosine (C) allele (C:C) on the rs2268493 (n = 11, M = 4.31, SE = 5.54) showed higher cortisol levels than participants who were heterozygous (C:T) (n = 83, M = 4.39, SE = 5.41) or homozygous for the thymine (T) allele (T:T) (n = 66 , M = 4.18 , SE = 5.41).

The third criterion for mediation was that cortisol (mediator) needed to be associated with vocal symptoms (outcome), and this was shown by the results in Study III. One SNP had an effect on cortisol and the association remained when both cortisol and the rs2268493 SNP were included in the model, indicating mediation. The effect of the SNP (rs2268493) on vocal symptoms was no longer significant when cortisol was included as a covariate in the model, however, the regression coefficients for C:C and C:T (genotype T:T as reference group) were estimated to non-zero, indicating some remaining variance explained by the SNP. It should be noted that only 160 individuals had information available both on cortisol levels and the rs2268493 SNP and that the statistical power was reduced as a consequence in comparison to the test of the effect of the SNP without including cortisol in the model (n = 408).

In summary, the results showed that teachers who felt stressed reported more vocal symptoms than teachers reporting little or no stress. Stress was also the one risk factor with the strongest connection to occurrence of vocal complaints; however, the results did not show a prominent difference between the risk factors. A positive association between possible stress markers and vocal symptoms was found in the population-based sample. Equally, a positive association to vocal symptoms was found when using the biological stress marker of salivary cortisol. After controlling for multiple tests, one polymorphism, the rs1587097 *AVPR1A* polymorphism was significantly associated with vocal symptoms. In addition, the results indicated that the nominal effects of one *OXTR* polymorphism (rs2268493) on vocal symptoms might be partly mediated by cortisol.

5 DISCUSSION

In this thesis, the role of stress in the occurrence of vocal symptoms was investigated. This was explored in two different samples; in a sample of occupational voice users of elementary school teachers, and in a population based sample. The association was studied using self-reported possible stress markers, the biological stress marker cortisol, and polymorphisms in the stress related genes *OXTR* and *AVPR1A.*

The main findings were that a) stress had a positive association with vocal symptoms in elementary school teachers b) occurrence of heartburn, reflux, tenseness or exhaustion showed a positive association with vocal symptoms in the general population c) higher salivary cortisol levels were associated with higher levels of vocal symptoms d) one *AVPR1A* polymorphism (rs1587097) was associated with vocal symptoms after controlling the significance level for multiple tests. In addition, the nominally significant effect of one *OXTR* polymorphism (rs2268493) on vocal symptoms might be partly mediated by levels of cortisol.

5.1 Association between stress and vocal symptoms

5.1.1 Association between stress and vocal symptoms in the sample of elementary school teachers

In the sample of elementary school teachers, those with vocal symptoms reported more stress than those with less or no symptoms. The positive association between stress and voice symptoms is in line with other studies investigating stress factors in teachers (Gassull et al., 2010; da Rocha & de Mattos Souza, 2013). Even though the correlation coefficient was similar for all the voice ergonomic risk fields, stress was the only risk factor that showed a significant positive correlation with both the VHI-score and the vocal symptoms measured with the 7-item voice questionnaire used in Study I.

Of the vocal symptoms measured in Study I, the Voice Handicap Index Physical Scale (VHI-P) showed the strongest connection to stress. This scale includes statements such as: *I run out of air when I talk, The sound of my voice*

varies throughout the day, *My voice sounds creaky and dry*, *I feel as though I have to strain to produce voice*, *The clarity of my voice is unpredictable*. Though the VHI total score and the VHI physical scale were significantly correlated to stress; the emotional scale did not show a significant correlation. This could indicate that stress had an impact mainly on the physical aspects of participants' voice or vice versa.

Vocal symptoms occurring at least weakly were more commonly reported in the sample of teachers than in the population based sample for three out of five vocal symptoms that were measured in both samples. These findings are in line with the results of Roy et al. (2004), where chronic, acute or reoccurring voice disorders were more common among teachers in comparison to the general population. The vocal symptoms that occurred significantly more often among teachers in the current thesis were tired voice, hoarseness, and throat clearing or coughing while talking. Teachers are exposed to many voice ergonomic risk factors in their work environment and have high occupational vocal loading. Teachers might endure with vocal symptoms and continue teaching with a dysphonic voice, which could also be indicated by the high prevalence of vocal symptoms in teachers compared to the population based sample. Additionally, the teachers might get used to a hoarse voice quality (Sonninen, 1970) and wait to seek help for their vocal complaints. The teachers' perception of their voice use and ability to teach might, however, be more complex. One statement in the VHI emotional scale claims that poor voice quality arouses a feeling of incompetence, and the VHI emotional scale correlated with poor working culture. These results might indicate that apart from the emotional responses deriving from the vocal symptoms themselves, it is also possible that some teachers' reactions may be directly connected to negative prevailing attitudes in the school environment.

The 7-item voice questionnaire including both physical and functional questions also showed correlations to stress. The association between stress and vocal symptoms in teachers could be reciprocal, with the voice symptom itself being the stressor or source of anxiety (McAleavy, Adamson, Hazlett, Donegan & Livesey, 2008). This might be the case especially regarding occupational voice users such as teachers, were poor voice quality or difficulty to make oneself heard, may cause a feeling of incompetence (McAleavy et al.,

2008). As the VHI and the 7-item questionnaire measures prevalence of vocal symptoms during two different time points, at the time of survey and during the last 12 months, the result could indicate that the occurrence of stress might be associated with both long-term and short-term vocal symptoms. Longitudinal studies are needed to be able to determine the nature and causality of the relationship.

In regard to voice ergonomics for occupational voice users, it is important to consider the changing teaching environment that teachers are exposed to. These changes involve for instance open classroom solutions and increasing small group activities (Shield, Greenland & Dockrell, 2010). These changes increase noise levels if necessary precautions are not taken regarding acoustics, reduction of noise and voice amplification. If the noise levels in the teaching environment increase, it might lead to additional stress, since noise can be both a stressor (Maschke, 2011; Wallen, Hasson, Theorell, & Canlon, 2012) and affect vocal effort and vocal quality (Lyberg Åhlander, 2011).

It is also worth mentioning, that voice quality influences the learning environment, and teachers' attitudes regarding the consequences of the vocal symptoms might not always be in accordance with the children's needs. For different reasons, teachers might continue teaching with a dysphonic voice and this affects children's ability to process spoken language and can result in underachievement. (Lyberg Åhlander, Brännström & Sahlén, 2015; Morton & Watson, 2001; Rogerson & Dodd, 2005). This is something that teachers, speech language pathologist and key decision-makers planning schools and teaching environments should be aware of.

5.1.2 Associations between possible stress markers and vocal symptoms in a population based sample

In the population based sample of Finnish twins and their singleton siblings, the possible stress markers exhaustion, being nervous in situations where one is required to talk and heartburn, were positively associated with all vocal symptoms. When looking at the vocal symptoms, feeling of muscle tension or lump in the throat was the only vocal symptom associated with all four stress markers.

The statement *Do you feel nervous or tense in situations where you are required to talk?* is an item possibly more linked to acute stress rather than to chronic stress. Many stress inducing tasks or tests measuring acute stress reactivity, for instance the Trier Social Stress Test (TSST; Birkett, 2011) involves public speaking. In Study II all six vocal symptoms were more frequently occurring among those who reported feeling nervous or tense in situations that required talking. This result corresponded with the results from the study by Van Lierde et al., (2009), which showed significant decrease in overall grade of vocal quality and increased breathiness during stress inducing conditions of public speaking. However, since it was not possible to control for which kind of situations the participants referred to, it could also be that the feeling of being nervous or tense was a result of the vocal symptoms or unpredictable vocal quality during speech.

In line with how exhaustion is described in the Allostatic Load Model (McEwen, 1998), the feeling of exhaustion reported in Study II, could be related to chronic stress. During chronic stress the response system is overtaxed due to repeated activation without recovery, and the HPA axis becomes dysregulated. The dysregulation involves chronically elevated cortisol levels, which has been associated with elevated blood pressure, metabolic disturbance and impaired immune function. These are all symptoms that to some extent could influence the vocal apparatus or result in vocal symptoms, as those seen in the results. In addition to the changes in the HPA axis involved in chronic stress or allostatic load, other stress-pathways in the body are also activated more frequently due to frequently occurring stress. These pathways consist of the sympathetic nervous systems (SNS) and the sympathetic-adrenal medullary system (SAM) involving secretion of epinephrine and norepinephrine. The SNS and SAM bring together a number of physiological changes that can influence phonation including a more rapid breathing, changes in cardiovascular function, and redistribution of blood flow from visceral organs to exercising muscles. Results from experiments in healthy participants show increased muscle activity in the vocal apparatus as a result of a stress inducing task of public speaking (Dietrich & Verdolini Abbott, 2012; 2014; Helou, 2014). Even though these results relate to acute stress, it seems reasonable to assume that the effects of acute stress influence

the vocal apparatus correspondingly, if the acute stress occurs frequently and there is not time for recovery.

The association between chronic stress and vocal symptoms has not previously been studied in a population based sample, so comparisons are difficult to make. Studies conducted on help seeking participants or participants diagnosed with a voice disorder, show corresponding findings regarding an association between muscle tension or muscle tension dysphonia and self-reported stress symptoms (Altman et al., 2005; Dietrich, et al., 2008; Seifert & Kollbrunner, 2006; Van Houtte, Van Lierde & Claeys, 2010) or assessed life difficulties (Baker et al., 2012).

According to a review by Mizyed, Fass and Fass (2009), individuals who have been exposed to prolonged life stressors are more likely to complain of symptoms of GERD. Acid in the larynx and pharynx may lead to a number of mucosal changes (review by Khan et al., 2006) possibly resulting in laryngeal symptoms such as sore throat, chronic hoarseness, chronic cough and throat clearing, and sensation of a foreign body or lump in the throat (Hamdan et al., 2001; Koufman, 1991) similar to those reported in Study II. However, as concluded in a more recent review (Schneider et al., 2016) even though literature to date has shown a relationship between LPRD and voice, the nature of that relationship is not quite clear. Additionally, the correlation between exhaustion and the two variables heartburn and reflux were small in the population based sample in Study II. Thus, given the data at hand, it is not possible to rule out that participants might have been experiencing symptoms of heartburn or reflux without being exhausted or feeling stressed.

5.1.3 The role of salivary cortisol in the occurrence of vocal symptoms

When using salivary cortisol as a biomarker of stress, the results indicated that participants with higher levels of cortisol experienced vocal symptoms more often than participants with lower levels of cortisol (i.e. higher levels of vocal symptoms as measured using the composite score). The vocal symptoms with association to cortisol were *Difficulty in being heard*, *Throat clearing or coughing while talking* and *Sensation of muscle tension or a lump in the throat*. The vocal symptom *Sensation of muscle tension or a lump in the throat*, was the only item in SCREEN6 that showed significant associations to both the stress markers

in Study II as well as to salivary cortisol levels in Study III. This might suggest that the association of vocal symptoms and cortisol levels could be related to higher allostatic load (McEwens, 1998) and result in laryngeal muscle hyperfunction as suggested by the Revised Psychobiological Framework of Voice Disorders (Helou, 2014).

Elevated cortisol levels have been associated with immunosuppressive changes and impaired wound repair (Christian et al. 2006; Ebrecht et al. 2004; Gouin & Kiecolt-Glaser, 2011). Hyaluronic acid is important in the healing process of laryngeal wounds (Branski, Verdolini, Sandulache, Rosen & Hebda, 2006) and animal studies have found an increase of degraded and activated hyaluronic acid in the tissues and blood in mice exposed to acute stress (Inoue et al., 2009). It could be speculated if elevated cortisol levels occurring due to allostatic load, and co-occurring with for example repair of vocal lesions, high vocal load, laryngopharyngeal reflux or vocal fold inflammation, might have associations to occurrence of vocal symptoms as those found in Study III. However, since neither the state of the larynx nor biochemical markers associated with wound healing were investigated, the possible connections to immunosuppressive changes are speculative.

When exhaustion was added as a covariate in the analysis regarding cortisol and the composite score of vocal symptoms, the association between cortisol and vocal symptoms was somewhat lower and no longer significant. The lack of significance was likely affected by lower statistical power due to missing values in the exhaustion variable and thereby a smaller sample size. Due to the reduction in statistical power together with overlapping confidence intervals for the size of the association between cortisol and vocal symptoms with and without exhaustion included, it cannot be excluded that cortisol could explain variation in vocal symptoms, not captured by the exhaustion variable. However, the nature of the cortisol measurements makes it difficult to draw conclusions regarding these findings. Using the cortisol awakening response, which involves multiple cortisol samples, could give more reliable information regarding the association between cortisol and vocal symptoms. The association between cortisol and vocal symptoms remained significant also when controlling for symptoms of anxiety and depression and this

suggested that the results seem specific to vocal symptoms instead of an overall tendency to report health complaints (Haug et al., 2004).

5.2 Gender differences in the association between stress and vocal symptoms

In line with several previous studies, vocal symptoms were more common among women in comparison to men in the population based sample, including the sub samples used in Studies III and IV (e.g. Coyle, Weinrich, & Stemple, 2001; Herrington-Hall et al., 1988; Roy, et al., 2005).

The results also showed gender differences in the occurrence of possible stress markers. Women and men have shown different patterns regarding experience of stress (Cohen & Janicki-Deverts, 2012), care seeking behavior as well as the perception of symptoms of stress (Lundberg, 2008). Exhaustion was more common among women in the present thesis, and women reported feeling nervous or tense in situations where they are required to talk more often than men. Women also had higher salivary cortisol levels than men, although this difference did not quite reach statistical significance. The results of the study by Weekes et al. (2005), showed that self-reported stressor exposure was a significant negative predictor of health for both men and women. However, the perception of stress was a negative predictor only for women. Previous findings suggest a greater cortisol awakening responses (CAR) in women than men (e.g., Weekes, Lewis, Goto, Garrison-Jakel, Patel & Lupien, 2008; Wüst et al., 2000b). This seems to be in line with the results from the current thesis, even though the nature of the cortisol measurements were not the same, which hampers comparisons.

No gender difference in the association between cortisol levels and vocal symptoms was found in Study III. The study needs to be replicated with a larger sample to be able to draw more conclusions regarding the effect of gender. The results by Reynolds et al. (2013) highlight a clear gender difference in HPA activity under non-stressful conditions. However, as mentioned by Hellhammer, Wüst, and Kudielka (2009) the influence of sex steroids on central HPA axis activity is rather complex and still only partially understood. In a study by Dietrich et al. (2008) investigating stress, anxiety,

and depression among 160 patients who presented with muscle tension dysphonia, benign-appearing vocal fold lesions, paradoxical vocal fold motion disorder, and/or glottal insufficiency, women were noted to have higher stress scores than men. Misono et al. (2014) found similar results; however, neither of these studies compared the link between stress and vocal symptoms between men and women.

5.3 Effects of polymorphisms in the *OXTR* and *AVPR1A* genes on vocal symptoms

An effect was found regarding three *OXTR* polymorphisms (rs2270465, rs2268493, rs7632287) using the nominal *p*-value threshold of $p = .05$. These SNPs did not remain significant after adjusting the *p*-value threshold to correct for multiple testing, so the results should be interpreted with caution and replication is needed. None of the nominally significant *OXTR* SNPs had previously been studied in relation to stress reactivity. However, all three polymorphisms have in previous studies been associated with social behavior (Westberg & Walum, 2015). The rs2270465 (Wermter, Kamp-Becker, Hesse, Schulte- Korne, Strauch & Remschmidt, 2010), rs2268493 (Campbell et al., 2011) and rs7632287 (Campbell et al., 2011; Tansey et al., 2010) have been associated with risk for autism and the rs7632287 has also been associated with pair-bonding (Walum et al., 2012). In a study by Kawamura et al. (2010) investigating affective temperaments, the results showed an association between depressive temperament and a specific *OXTR* haplotype (set of alleles on one chromosome), where the rs2268493 was as one of the seven polymorphisms in the haplotype. The gene-based association analysis showed no significant effects of the *OXTR* and the *AVPR1A* on vocal symptoms, however, a better coverage of genotyped polymorphisms of the respective genes would have yielded a more powerful gene-based test.

In addition, the results indicated that the nominal effect of the *OXTR* rs2268493 on vocal symptoms might be partly mediated by cortisol levels. The mediational analysis was conducted to investigate whether possible effects of the polymorphisms on vocal symptoms could be mediated through cortisol, given the involvement of cortisol, oxytocin and vasopressin in the psychoneuroendocrinology of the stress reaction. A contributing factor to the

mediation analysis was the reduction in statistical power arising from partly different samples being used in the two steps. Only 160 individuals had information available both on cortisol levels and the rs2268493 SNP, in comparison to the test of the effect of the SNP on vocal symptoms without including cortisol in the model (n = 408).

Two potential pathways could be hypothesized for the association between *OXTR* and vocal symptoms that could act separately or in combination: 1) the SNP could through its potential association with oxytocin function, physiologically affect cortisol levels, which in turn would influence vocal symptoms or 2) the SNP could through its association with social traits influence for example seeking of peer support during stress (Chen et al., 2011), which in turn could alleviate stress symptoms (through the release of OXT), this in turn affecting risk for vocal symptoms. Thus, there might exist a genetic predisposition to become stressed, which could influence the occurrence of vocal symptoms – or there might be a genetic predisposition to cope with stress in a certain way or seek support when stressed, that could influence the occurrence of vocal symptoms. Of course, genes are likely to influence vocal symptoms directly as well without any association to stress, however, *OXTR* and *AVPR1A* were theoretically chosen as candidates for genetic influences on vocal symptoms due to the previously known associations between OXT and AVP and stress.

Of the two nominally significant *AVPR1A* polymorphisms (rs1587097, rs1042615), the association between *AVPR1A* rs1587097 and vocal symptoms remained significant after correction to account for multiple testing, with cytosine (C) being the potential risk allele. These polymorphisms have to date not been analyzed in a relation to stress, however, Levran et al. (2014) identified rs1587090 as one polymorphism with nominally significant effects on substance abuse, when analyzing effects of stress-related genes on substance abuse.

5.4 Methodological considerations

The findings, results and conclusions in Study I might have been affected by selection bias (Kahan, Rehal, & Cro, 2015). During the assessment and data

collection the participants (N = 39) also provided other research data connected to the project *Acoustics and voice ergonomics in classrooms – from investigation to intervention*, and this might have meant that only interested teachers took part. The participating teachers might have been either more aware of their voice to begin with or may have been experiencing vocal complaints, motivating them to contribute to research in this specific field. Another factor that may have influenced the randomization of the data in Study I, was that the researchers who made the voice ergonomic assessments were members of the research group. This setting was used since the VEAW assessment method requires training, which only very few voice experts had at the time of the data collection. Moreover, a larger sample size would have increased the reliability of the results.

In Studies II-IV, a population-based sample of Finnish men and women participated. Even though the sample consisted of twins as well as their siblings, generalizability of the results is not limited only to twins. Studies have shown that there are rarely any differences between twins and singletons, exceptions being weight (Andrew, Hart, Snieder, de Lange, Spector & MacGregor, 2001) complications during birth and language development (Rutter, 2006). Furthermore, the GSA-sample is comparable to another population based sample of the general population in Finland with regards to experiences of childhood maltreatment and sexuality related items (see Albrecht, Antfolk, Liberman, Harju, Sandnabba & Santtila, 2014), indicating that the sample is representative of the general population. The response rates for the questionnaire data were somewhat low, 53% and 21% respectively for the two data collections used and it might not be fully representative for the population. The lower response rate in the second data collection might have been influenced by the participants being asked to answer the online questionnaire on speech, language and voice, after having already filled in in an extensive questionnaire. Furthermore, participants were recruited amongst those who had responded to the GSA-questionnaires addressing sensitive topics related to sexuality and aggression, which might have affected the representativeness of the sample. However, using participants leaving the last third of the second GSA-online questionnaire unanswered as a proxy for non-responders, slight differences between

responders and non-responders were seen only for a few sexuality related variables (see Johansson et al., 2013, for details), and it is unlikely that such differences would have an influence on the representativeness of voice-related phenotypes.

All four studies were based entirely or partly on self-report questionnaire data. Using self-report retrospective questionnaire makes the data susceptible to recall and response bias. Experimental study designs allow for a test of the causal effects of, for example, an acute stressor on voice while controlling for other situational factors, but the generalizability of the results might not be as good to naturalistic settings as from studies using large self-report questionnaire data. In this thesis, cortisol levels were used in addition to self-reported exhaustion, and the results regarding the association to vocal symptoms were comparable between both methods. The use of questionnaire based self-reports also makes it easier to collect data from large population based samples.

The assessment of vocal symptoms in Study I was done using a 7-item voice questionnaire and VHI, and in Studies II-IV using the SCREEN6. Using two voice questionnaires with slightly different focus in Study I, one being an internationally widely used and validated measure of voice handicap, made the data more reliable. Even though the SCREEN6 has been widely used in voice research (Fellman & Simberg, 2016; Hagelberg & Simberg; 2015; Nybacka, et al., 2012; Simberg, et al., 2004; Simberg, et al., 2006; Simberg et al., 2009) and compared in connection to vocal quality (Simberg & Sala, 2008) and laryngeal examination by a laryngologist (Sala et al., 2001), the SCREEN6 has not yet been validated. Moreover, the vocal symptoms included in the study do not only reflect the vocal function of the speaker, some are also strongly related to environmental factors such as background noise (for example *Difficulty in being heard*). It could also be argued that *low in pitch* and *hoarse* that is used in the statement *Voice becomes hoarse or low in pitch* (SCREEN6), are not synonymous but should be regarded as to different vocal symptoms and as such should form two different items in the screening tool. Additionally, as the name inclines, the question set is designed to work as a screening tool, and using a more detailed set of questions might have yielded more information.

However, using extensive questionnaires might on the other hand result in lower response rate.

When investigating the influence of stress on voice, the time perspective has varied greatly between studies. Changes in vocal acoustics (Mendoza & Caballo, 1998; Mendoza & Caballo, 1999) or extralaryngeal reactivity (Dietrich & Verdolini Abbott, 2012, Dietrich & Verdolini Abbott, 2014) due to stress, have been measured mainly during acute stress protocols. Questionnaire studies regarding vocal symptoms and stress have been conducted to some extent, and in these studies, the questions regarding stress levels or perceived stress have involved various time periods. The time periods have varied from no time limit for example "Have you had the following diseases? Stress: Yes/ No" (Chen et al., 2010) to present time with evaluation several times during the day on a scale from 1 to 5 (Lyberg Åhlander, Pelegrín García, Whitling, Rydell & Löfqvist, 2014). In the Perceived Stress Scale with ten (PSS-10) or four items (PSS-4), which are widely used tools to measure global perceived stress in relation to health-related outcomes (Dietrich et al., 2008; Misono et al., 2014) the respondent is to evaluate feelings and thoughts during the last month. Stress over a longer period of time (12 moths) and voice has been investigated by Baker et al., (2012) using the semi-structured interview Life Events and Difficulties Schedule (LEDS). In questionnaire studies, the participant's interpretation of the question and his or her current stress levels and current sense of well-being may influence the results. Even though the recall bias is a problem in all study designs using self-report questionnaire, it has long been argued that the recall bias is particularly problematic for research on stress, coping and mood. Recall on past mood, past positive and negative events or past stress levels are influenced by the mood at the time of recall (review by Stone & Shiffman, 1992). In this thesis, all self-report questions regarding stress and exhaustion were set at the time point of the day of the survey, which could be seen as a precaution for recall bias, even though the time point compared to the occurrence of vocal symptoms was different (vocal symptoms during the last 12 months).

Regarding the possible stress markers used in Study II, exhaustion showed a small positive correlation to all other stress markers, the exception being diagnosed reflux among women. This exception might be affected by lower

statistical power due to smaller sample size, the prevalence of reflux in the sample being only 4%. Since exhaustion was the one of the four stress markers with possibly the strongest connection to feeling stressed (McEwen, 1998; Lundberg; 2002), the fact that this item showed a correlation with all the other three stress markers, supported the use of the included items as markers of stress. However, as mentioned, the correlations were small. This might partly have been influenced by the dichotomous nature of the four stress markers. Using a questionnaire with Visual Analogue Scale or Likert scale based response alternatives and a more extensive questionnaire regarding stress would have increased reliability (Hasson & Arnetz, 2005). Regarding the symptoms of heartburn and reflux, there are numerous studies reporting LRPD or GERD possibly being secondary to or associated with stress (Cassileth & Drossman, 1993; Drossman, 1996; Morrison et al., 1999; Núñez-Rodríguez & Sivelo, 2008). However, heartburn and reflux could of course also occur without the person feeling or being stressed and without consequences for vocal function or in voice quality (Schneider et al., 2016).

Using saliva samples is a noninvasive and objective method of measuring stress, which also makes it possible to reach and study a larger group of participants. However, there exist challenges regarding the stress measurements used in this thesis and data collection. The cross-sectional nature of this thesis is a limitation and using multiple cortisol samples would have given the authors a more reliable picture of how stress is related to vocal symptoms. Kristenson et al. (2011) report that the correlation between cortisol levels on consecutive days has been reported to be around $r = 0.5$, which is why mean levels over two or three days often are used to give more reliable results. As most studies understandably use multiple data points when investigating life stress or perceived psychological stress, it is difficult to reliably compare the results with results from other studies. Furthermore, stress (acute stress or moderate duration of chronic stress) is associated with elevated morning cortisol levels (for example Clow, Thorn, Evans & Hucklebridge, 2004) whereas burnout is associated with hypocortisolism (Lennartsson et al., 2015), and using CAR and an assessment of exhaustion could have made further information about the relationship between stress and vocal symptoms.

The stress system is affected by changing levels of sex hormones, as found for instance in the premenstrual period, ante- and postpartum, during the transition phase to the menopause and during the use of oral contraceptives (Swaab et al., 2005). Reynolds et al. (2013) found that cortisol levels were significantly reduced by oral contraceptive use. In the current thesis, we could not control for contraceptive use, phase in menstrual period or phase in menopause among the female participants. Nor could we control for the exact time point after awakening of the salivary sample. However, in a study by Wüst et al., (2000b) the authors concluded that neither age, time of awakening nor use of contraceptives have a considerable impact on free cortisol levels after awakening. There are of course confounders regarding measurements of cortisol in saliva, and even results indicating that perceived stress can be expected to be only moderately associated with salivary cortisol (Hellhammer et al., 2009), however; hormone samples can be a useful objective indicator of stress in the individual.

With regard to the Study IV, it is important to mention that the results regarding the polymorphisms showing association with vocal symptoms could be affected by the SNPs being in LD with other functional variants affecting *OXTR* or *AVPR1A* function, and the functionality of these SNPs are still unknown. Additionally, in the sample only three persons were homozygous for the C allele (C:C). Since this was a too small number to analyze separately, they were grouped together with the heterozygotes (C:T). It is therefore not possible to determine whether or not the effect of the C allele on vocal symptoms is affected by dominance. Replication of this study is needed, as well as identifying the specific pathways in which OXT and AVP influence vocal symptoms.

Lastly, the presence of significant associations reported in the cross-sectional study design used in the four studies, does not of course imply a relationship of cause and effect. As described in the review by Baker (2008) there are several models that could be implemented for describing the relation between stress and vocal symptoms leading to vocal pathologies; introvert personality traits (Dietrich & Verdolini Abbott, 2012; Dietrich & Verdolini Abbott 2014; Roy & Bless 2000a; Roy & Bless, 2000b), stress reactivity (Dietrich & Verdolini Abbott, 2012), negative emotions following stressful life events (Baker et al.,

2012; Seifert & Kollbrunner, 2006), conflict over speaking out (Baker et al., 2012) and coping strategies (Baker 2012; Baker, Oates, Leeson, Woodford & Bond 2014; Zambon et al., 2014), are thought to influence the etiology of functional voice disorders. There might also exist other contributing effects, such as noise sensitivity (Maschke, 2011; Wallen et al., 2012) where noise can be both a stressor and affect vocal effort. As discussed in an epidemiology study by Brainbridge, Roy, Losonczy, Hoffman and Cohen (2016) hypertension is itself associated with stress, which may contribute to vocal pathologies (Giddens et al., 2013). In addition, side effects caused by hypertension-medication, such as angiotensin-converting enzyme inhibitor-induced cough might also be related to voice disorders. Because other factors such as level of personality, coping strategies, allergies, leisure activities, habits, and environmental factors were not taken into account, based on results from the current thesis it can be concluded that stress might be one factor in the multifactorial etiology of vocal symptoms.

5.5 Conclusions and suggestions for future research

A positive association between stress and vocal symptoms was observed in the sample of occupational voice users of elementary school teachers, as well as in the population based sample. The association between stress and vocal symptoms has not previously been studied in a large population based sample. Other novel elements in this thesis comprise of the biological aspects of the stress hormone cortisol and the genetic variants in the stress related genes *OXTR* and *AVPR1A*.

Voice symptoms were common among teachers, and apart from stress, the occurrence of vocal symptoms was connected to many of the other risk factors included in the Voice Ergonomic Assessment in Work Environment – Handbook and Checklist. Although multiple risk factors affecting the voice are known, there has been no notable progress in the field of voice ergonomic assessment and intervention during the last decades. If anything, vocal symptoms have increased (Simberg, 2006) and voice ergonomic assessment was not included in any protocols of health care in any country 15 years ago (Vilkman, 2001), nor is it to the best of the author's knowledge included now. Future research could focus on the consequences of poor voice ergonomics for

the teachers, the pupils and for private and public economy, to possibly substantiate the benefits of an efficient voice ergonomic assessment and intervention.

Though the association between cortisol and vocal symptoms was weak, the result is significant, as cortisol is a biomarker of the physiological stress response without having to rely on self-report when measuring stress levels. Using hormone sampling or analyzing genetic risk factors in speech, voice and communication science is increasing, and additional data on the potential relevance of stress hormones and stress related genetic variants would help researchers design future studies.

Despite small effects of SNPs, identification of novel variants might lead to biological insights of relevance to the trait. The results of the effects of *OXTR* and *AVPR1A* polymorphisms should, however, be interpreted with caution. The specific association between the receptor genes and oxytocin or vasopressin levels in the brain and in the body, as well as the association of these hormones and glucocorticoid reactivity during stress is not yet fully understood. Research regarding additional stress related SNPs and replication is needed to identify the specific pathways involved. Since other factors, such as means of social support (OXT) and aggressive behavior (AVP), also might influence the relationship between OXT, AVP, stress and cortisol, it would be advisable to include these factors in future research. Possible gender differences could also be further explored, since there is evidence that *OXTR* and *AVPR1A* could predict gender specific emotional responses to acute stressors (Moons et al., 2014). Many of the genes that have been associated with the stress reaction or stress reactivity, are connected to the neuroendocrinology affecting the body and the brain during stress. This is also the case for genetic variants in the gene coding for the glucocorticoid receptor (review by Koper, van Rossum & van den Akker, 2014) which binds cortisol in the human brain (de Kloet et al., 2005). Thus, other genes might be fruitful to investigate in the connection to vocal symptoms and disordered voice and besides the use of a theoretically driven candidate gene approach, as in the current thesis, explorative genome-wide association studies might prove fruitful in identifying novel genetic variants influencing voice

symptoms when large enough sample sizes are available. Future research could further explore clinical implications. This could involve defining groups based on vocal symptoms and estimate the effect of genotypes and cortisol in clinically relevant groups. This type of research would increase our understanding of how the oxytocin and vasopressin system affects the stress response and risk for vocal symptoms.

Furthermore, longitudinal studies including both acute and chronic measurements of stress in a population based sample could increase the means of determining the nature of the relationship. Increased knowledge on the etiology of vocal complaints and on reasons for the variation in vocal symptoms between individuals, will further improve preventive voice care. Furthermore, it will enable continued development and implementation of effective and evidence based intervention in voice clinics, hopefully ensuring good voice health both for occupational voice users and for the general population.

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